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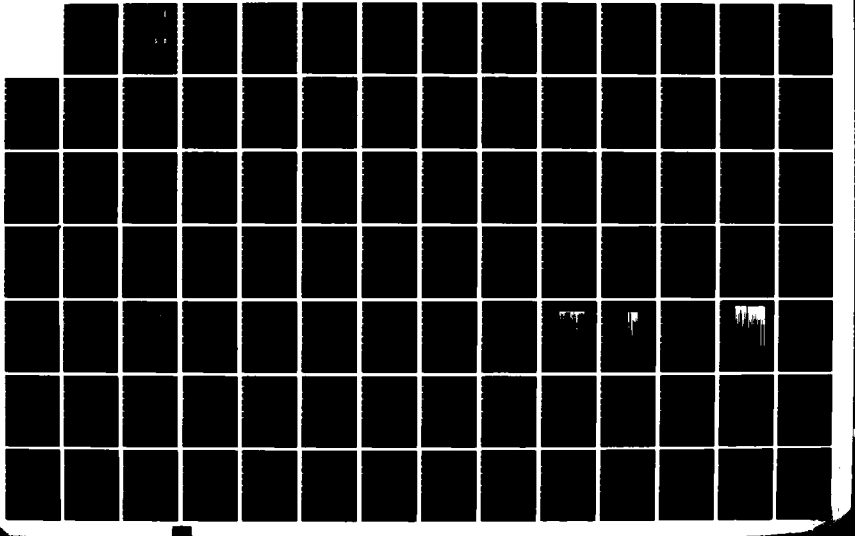

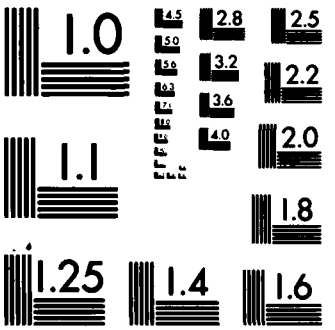

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ANNUAL PROGRESS REPORT

Robert E. Shope, M.D.

February 1981

Supported by

U.S. Army Research and Development Command, Fort Detrick,
Frederick, Maryland 21701

Contract DADA-17-72-C-2170

Yale University School of Medicine
New Haven, Connecticut 06510

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Virus Identification. Soldado Rock virus was identified from ticks of the Seychelles; Pongola, a new Yogue group virus from bats and human febrile cases, and an apparently new bunyavirus were identified from Uganda; a strain of West Nile virus from an apparently rabid dog in South Africa was identified; from Australia, a new tick-borne flavivirus was characterized (isolated from ticks of birds; Ross River virus was confirmed from serum of a case of epidemic polyarthritis and rash in Fiji; four strains of Sindbis were identified from

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Lake Nasser, Egypt; and six strains of dengue, type 4 as well as other as yet unidentified viruses from New Caledonia were studied. An isolate of Colorado tick fever was identified from human serum of the Netherlands in a traveller.

Virus taxonomy. The Sakhalin serogroup was shown to belong in the Nairovirus genus. The IFA test reactions and cross-reactions of Rift Valley fever and other Phlebotomus fever group viruses indicated that the IFA test is relatively specific.

Serologic surveys. Surveys of Ghana, Liberia, Cameroon and the Sudan showed widespread activity of arboviruses and indication of activity of Lassa virus and Ebola virus in several areas of Africa.

Development of techniques. The ELISA and ELFA techniques were adapted to measure antibody to yellow fever, California group viruses and Rift Valley fever. Antigen could be detected in mosquitoes infected with Guaroa and La Crosse viruses indicating this technique may be applicable to detect arboviruses in field-caught mosquitoes.

Biochemistry. Most orbiviruses were found to have 10-segmented genomes; surprisingly, Colorado tick fever was discovered to have 12 RNA segments. It may, therefore, belong to another taxonomic set different from the other orbiviruses as they are now known.

Distribution of reagents. As in previous years, the reference center distributed large numbers of cell lines, viruses, antigens, and sera on a world-wide basis.

Tests of reassortant Phlebotomus fever group viruses for enhanced virulence. Enhanced virulence was not found for cloned prototype Phlebotomus fever group viruses or their reassortants.

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SUMMARY

Virus Identification. Two viruses isolated from Ornithodoros capensis and one virus from Rhipicephalus sanguineus ticks collected in the Seychelles Islands have been tentatively identified as Soldado Rock virus. Another virus has been isolated from Hyalomma a. anatolicum ticks collected in Bahrain and is currently being identified.

Four different viruses from Uganda were studied. An isolate from mosquitoes was identified as Pongola; an isolate from a fruit bat and from 4 human febrile cases was related to Yogue virus and is a new member of that group; an isolate from Amblyomma variegatum ticks was bunyavirus-like by electron microscopy but did not react serologically with other African viruses tested; and another isolate from human serum was chikungunya virus.

An interesting virus from brain of a South African dog suspected of rabies was West Nile virus, and another South African isolate from mosquitoes was Uganda S.

An agent from Ixodes uriae ticks of Macquarie Island, Australia was a new member of the group B tick-borne encephalitis complex, the first such member from south of the equator. Umbre virus of the Turlock group was identified for the first time from mosquitoes of Australia. Another virus from the serum of a patient in Fiji was closely related to Ross River virus, the cause of epidemic polyarthritis and rash.

Two new viruses from Culex mosquitoes from Brazil belonged to the Corriparta group. This is the first recognition of this group in the Americas.

A virus from soft ticks collected near Cape Frehel, France was a subtype of Soldado Rock virus.

Four viruses from mosquitoes collected near Lake Nasser, Egypt were identified as Sindbis virus.

Six viruses from mosquitoes of New Caledonia were group B agents, at least one of these is dengue, type 4; nine additional isolates from mosquitoes and birds have been established but not yet grouped.

An isolate from human serum from a febrile patient bled in the Netherlands was Colorado tick fever virus. The patient had vacationed in the western U.S.A. and returned sick to Holland where he removed a tick from himself. This is an example of long-distance transport of a human viral pathogen.

An agent recovered from Dermacentor variabilis ticks in Canada was negative in testing with sera to 87 viruses; another agent from Ornithodoros maritimus ticks collected in herring gull nests in Morocco was identified as a virus closely-related to Chenuda of the Kemerovo group.

Virus taxonomy. Members of the Sakhalin serogroup were shown to be related by HI and fluorescent focus neutralization tests, and to belong to

the Nairovirus Supergroup. Multiple viruses in the supergroup and corresponding reference sera were supplied to the University of Alabama where studies of RNA and proteins showed that these viruses formed a new genus in the family Bunyaviridae.

Serologic surveys. Surveys were conducted with sera from Ghana, Cameroon, Sudan, and Liberia using the IFA test. Sera positive for Ebola and Marburg antibody were found in Ghana. The Ebola positive sera were collected one year before the first recorded Ebola outbreak. Five of 41 sera from Cameroon were positive also for Ebola and 16/196 human sera from Liberia had Lassa virus antibody.

Sera from Sudan contained IFA antibodies to West Nile, group A (probably Sindbis and chikungunya), Rift Valley fever, group Bunyamwera (probably Bunyamwera, Germiston, and Ilesha), Tataguine, Quaranfil, Bwamba, Tahyna, Bangui or Zinga, and Sicilian sandfly fever viruses. Antibody to Ebola virus was also found.

Development of techniques and models. In further development of the use of RNA purification and gel electrophoresis, it was found that Colorado tick fever (CTF) virus contained 12 segments, 2 more than found in any other orbivirus. This surprising result may mean that CTF virus, believed to be an orbivirus, is in a different genus of the Reoviridae family.

The IFA test has proved rapid, sensitive, and for some families of viruses, widely cross-reactive. This test offers the possibility of rapid, inexpensive virus identification and survey for antibody. The technique was developed and tested for members of a number of serological groups of arthropod-borne viruses. Within arbovirus groups A, Bunyamwera, and PHL, the test was broadly cross-reactive and as such was useful as the first test of broad serological surveys.

The ELISA was developed for detection of antigen in mosquitoes and the ELISA and ELFA for detection of RVF antibody.

Distribution of reagents. The reference center distributed 668 ampoules of reference sera, viruses, and antigens during 1980; mosquito tick and vertebrate cell lines were also distributed. Of the viruses and immune reagents distributed, there were represented 196 different arboviruses.

FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

I. VIRUS TAXONOMY

Nairovirus Genus (J. Casals and G.H. Tignor). Evidence was given in the 1979 annual report that several antigenic groups of virus in the Bunyavirus-like and unclassified categories were distantly, but reproducibly related by several tests, particularly the HI test. These observations, showing the existence of a Nairovirus "supergroup," supplied the leading basis for the establishment of a new genus, Nairovirus, in the family Bunyaviridae. The antigenic groups thus linked were: CCHF, Nairobi sheep disease, Dera Ghazi Khan, Hughes and Qalyub.

Two tests with one sample of Sakhalin virus gave low titers by HI, 1:5 or 1:10, against CCHF and Dugbe antigens; this was considered insufficient evidence to justify incorporation of the Sakhalin group in the Nairovirus genus, unless it could be confirmed.

A new immune serum was prepared with Sakhalin virus and mice were also immunized with Avalon virus, another member of the Sakhalin group. These reagents were tested by HI against CCHF, Dugbe and Hazara antigens, with the result shown in Table 1. In view of these results it appears justifiable to include the Sakhalin group in the Nairovirus "supergroup"; biochemical investigation, to be carried out in another laboratory, will determine whether other properties warrant the inclusion of the Sakhalin group in the Nairovirus genus.

Serological relationships among members of the Phlebotomus fever group of viruses as determined by immunofluorescent assay (J. Meegan and D. Winograd) The immunofluorescent (IF) test has proved rapid, sensitive, inexpensive, and for some families of viruses, widely cross-reactive. We have explored the cross-reactivity of the IF test using viruses of the Phlebotomus fever groups (Phlebovirus), alphavirus, and bunyavirus to determine the applicability of IF as the initial screening test in serological surveys. Additionally, considering the growing importance of the IF test in the diagnosis of disease, it was important to understand the cross-reactions between human pathogenic viruses within serological groups.

Tables 2, 3, and 4 show the results to date. A comparison of the IF relationships in the Phlebotomus fever group with those determined by the hemagglutination-inhibition (HI), complement-fixation (CF) and neutralization (N) tests shows no consistent agreement. However, in all except a few cases there is either an HI, CF, or N cross-reaction wherever there is an IF cross. A notable exception is the reaction of antisera to Rift Valley fever (RVF) virus with sandfly fever Sicilian antigen. Since these viruses circulate in the same geographic area in northern Africa, this cross-reaction represents a diagnostic dilemma.

Table 1

Inclusion of the Sakhalin group of viruses in the Nairovirus genus
Hemagglutination-inhibition test

Serum	Antigen, number of units				
	CCHF		Dugbe		Hazara
	16	8	4	2	16
Sakhalin, a	5	10	10	20	0
b	10	20	10	20	0
Avalon	20	40	20	40	5
Dugbe	160	160	160	160	80
CTF	0	0	0	0	0
Mono Lake	0	0	0	0	0
Bwamba	0	0	0	0	0
Oropouche	0	0	0	0	0

Reciprocal of serum titer; first dilution, 1:5.

Table 2

Immunofluorescence (indirect) reactions among Phlebotomus fever group viruses

Antibody	Antigens (spot-slides)											
	RVF	PT	CDU	ARU	SFS	ITP	SAF	ANH	FRI	NIQ	GOR	SFN
RVF-rab[1]	-	4	0[2]	4	16	16	32	0	32	32	4	0
RVF-hum[3]	512	8	8	8	64	32	64	0	256	64	4	0
PT	-	2048	0	8	0	32	8	0	16	128	0	0
CDU	-	0	256	0	0	0	0	0	0	16	0	0
ARU	-	0	0	256	0	0	0	0	4	0	0	0
SFS	-	0	0	0	128	0	0	0	0	0	0	0
ITP	-	0	0	0	0	256	0	0	16	0	0	0
SAF	-	0	0	0	0	8	256	0	16	0	64	0
ANH	-	8	8	0	0	0	0	512	16	0	0	0
FRI	-	0	0	0	4	0	16	0	256	0	0	0
ICO	-	32	0	0	0	32	8	0	32	16	0	0
GOR	-	16	8	0	4	32	64	0	16	8	1024	32
SFN	-	0	0	8	0	16	128	0	32	16	4	1024

[1]Rabbit inoculated with live virus. We have found less cross-reactions when using mouse immune sera prepared by inoculation of inactivated virus.
[2]0=<4. Titers expressed as reciprocal of serum dilution.
[3]Human convalescent sera.

Table 3

Immunofluorescence (indirect) reactions among alphaviruses

Antisera*	Antigen (spot slides) CHIK	ONN	SFV	MAY	SIN
CHIK	256	16	8	8	0
ONN	0	32	0	0	0
SFV	128	16	1024	512	16
MAY	16	32	32	512	32
SIN	0	0	8	0	256
WEE	0	0	0	0	32
VEE	16	>32	>32	16	>32
EEE	4	0	0	0	0
MID	0	0	4	0	0

*Mouse ascitic fluid, 0 = <4.

Table 4

Immunofluorescence (indirect) reactions among Bunyaviruses

Antisera*	Antigen (spot-slides) BUN	GER	ILE	BWA	TAH
Bunyamwera	512	8	32	0	0
Germiston	32	256	64	0	0
Ilesha	32	0	64	0	0
Bwamba	0	0	0	64	0
Tahyna	0	0	0	0	16

*Mouse ascitic fluid, 0 = <4.

II. VIRUS IDENTIFICATION

ALPHAVIRUS

Fiji-41451. (B.Q.Chen, S.M. Buckley and J. Casals). The initial steps on the identification of this strain were reported in the 1979 Annual Report. The virus had been isolated by Dr. R. B. Tesh from the serum of a patient seen in the course of an epidemic of polyarthritis in that island.

The agent could not be adapted to propagation in new born mice; hemagglutination antigens were prepared from Vero and BHK-21 cell cultures infected with the strain. An immune serum was prepared in mice by repeated inoculation of Vero-cell-propagated viruses. With these reagents, the Fiji strain was identified as Ross River virus or an agent very similar to Ross River virus, Tables 5 and 6.

EgAr 989, EgAr 996, EgAr 1003, and EgAr 1019 (R.E.Shope). Seven viruses isolated from Culex pipiens mosquitoes and one from Anopheles spp. collected in Aswan Governorate were supplied by Dr. Rifky El-Karamany and Dr. Imam Z. Imam of Cairo, Egypt. Four of the isolates were not viable. The other 4 were established in mice. In reciprocal cross-CF tests, these isolates reacted to titer with Sindbis antigen and antibody.

FLAVIVIRUS

CS-122 (A. Brescia and J. Casals). Identification of this strain, initiated in 1979, has now been completed. The strain was isolated by Dr. T. St.George, CSIRO, from Ixodes uriae nymphs collected on Macquarie island, in 1976. The strain was easily confirmed as being a group B virus by complement fixation (CF) and indirect immunofluorescence (IF) tests. Cross-CF tests showed that CS-122 was closer to the tick-borne group B viruses than to other flaviviruses, but easily distinguishable from them. The result of a CF test is shown in Table 7; in addition a CS-122 hyperimmune mouse serum gave negative reactions at dilution 1:8 with the following antigens: Banzi, dengue 2, dengue 4, Edge Hill, Kadam, KFD, Kokobera, Kunjin, MVE, Saboya, Sepik, SLE, Usutu and yellow fever.

CS-122 was compared with a strain of Central European Tick-borne encephalitis virus (ACA), in an intracerebral mouse neutralization test with the results shown in Table 8. In view of the CF and neutralization test results it is tentatively concluded that strain CS-122 is a new flavivirus, closer to some of the members of the tick-borne virus complex than to the remainder flaviviruses.

SA AN - 2 (Calunda and A. Mukuye). The strain was submitted by Dr. B. McIntyre, Pringham, South Africa; it had been isolated from the brain of a dog which presented a rabies-like disease.

SA AN-24630 strain propagated easily in Vero and BHK-21 cell cultures with CPE appearing in 2 or 3 days; the strain killed newborn mice in 3 or 4 days. The strain was shown to belong in antigenic group B by testing

Table 5
Identification of Fiji-41451 strain
Hemagglutination-inhibition test

Antigen, 8 units	Serum Fiji-41451
Fiji-41451	1600
EEE	0
WEE	0
VEE	0
chikungunya	0
Ross River	200
Getah	0

Reciprocal of serum titer; 0, negative reaction at dilution of serum 1:50.

Table 6

Identification of Fiji-41451 strain
Hemagglutination-inhibition test

Serum	Antigen, 8 units	
	Fiji-41451	Ross River
Ross River, a	160*	160
Ross River, b	80	80
Ross River, c	640	640
Fiji-41451, a	160	80
Fiji-41451, b	2560	640

*Reciprocal of serum titer.

Table 7

Identification of strain CS-122
Complement-fixation test

Antigen	Serum					
	CS-122	CETBE	TYU	LAN	ZIKA	WN
CS-122	512/256	32/32	4/2	32+/32+	8/32	16/16
CETBE (ACA)	32/64	256/256				
Omsk HF	4/16+					
Tyuleniy			32+/32+			
Langat	16/32+			32+/32+		
Louping ill	8/16					
Zika	8/4				32+/32+	
West Nile						32+/32+
Tembusu	8/4					

Reciprocal of serum titer/reciprocal of antigen titer.

Table 8

Identification of strain CS-122
Neutralization test by IC Route in Mice

Serum	Virus			
	CS-122		CETBE	
	$ICLD_{50}$	log NI	$ICLD_{50}$	log NI
CS-122	$10^{-3.9}$	2.7	$10^{-6.7}$	-0.4
CETBE (ACA)	$10^{-5.5}$	1.1	$10^{-3.7}$	2.6
Control	$10^{-6.6}$		$10^{-6.3}$	

NI, neutralization index

infected BHK-21 cultures against the following polyvalent grouping immune ascitic fluids by the IF test: groups A,B,C, Bunyavirus, Bwamba, California, Capim, Congo, Guama, Kemerovo, Palyam, phlebotomus fever, Quaranfil, rabies, Simbu, Tacaribe, VSV, and polyvalents #1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 (NIH, Catalogue of Research Reagents). A strong positive reaction was given by the group B ascitic fluid while all others were negative.

An immune mouse serum against SA AN 24630 prepared in South Africa, was tested by CF test against antigens derived from 47 different flaviviruses; the serum was tested in increasing two-fold dilutions beginning at 1:8 and the antigens were used in dilutions 1:4 and 1:16. The result of the test is given in Table 9. A second CF test confirmed the results shown in Table 9, and indicated the close relationship between SA AN 24630, West Nile and Kunjin. Since the latter has never been isolated in Africa, it was tentatively concluded that SA AN 24630 was a strain of West Nile; the result of a final CF test, Table 10, appears to confirm the conclusion.

SA Ar 19786 virus (I.Marika, G.Roze, and R.E.Shope). This virus was received from Dr. B. McIntosh of the South Africa Institute for Medical Research. It reacted by CF with the group B grouping fluid. A screening CF test detected cross-reactions with Usutu, Uganda S, yellow fever, and dengue 1 viruses; the following were negative: Banzi, Spondweni, Zika, Wesselsbron, Dengue 2 and 3, Ntaya and Kadam. A hemagglutinating antigen was prepared with optimum reactivity at pH 6.4-6.8. An HI test indicated a very close relationship of SA Ar 19786 virus to Uganda S (Table 11) and distinctness from Usutu, yellow fever, and dengue 1.

NC Ar 250 (R.E.Shope, P. Garbe, and G. Roze). Twenty virus strains were received from Dr. P. Fauran of the Institut Pasteur, Noumea, New Caledonia for identification. Five of these were not viable in mice. Of the others, NC Ar 250, NC Ar 254, NC Ar 262, NC Ar 264, NC Ar 281, and NC Ar 323 reacted with the group B grouping fluid by CF test. NC Ar 250 was studied further as a representative of these flaviviruses. Positive CF reactions were found with dengue viruses, types 1, 2 and 4. Nine other flaviviruses of Australia and Asia were negative. Reciprocal CF tests with dengue 1,2 and 4 showed strongest reactions with dengue, type 4 (Table 12). NC Ar 250 was thus presumptively identified as dengue virus, type 4.

Of the other New Caledonia isolates, NC Ar 238, NC Ar 259, NC Ar 265, NC Ar 267 and NC Ar 318 killed mice in less than 2 days. Identification of these is incomplete. NC Ar 280, NC Ar 309, NC Ar 318, and NC An 1 had incubation periods in mice greater than 2 days, did not react with the group B fluid, and remain unidentified.

Dak Ar D 14701 (T. Jamnback, G. Roze, and R.E.Shope). This virus was referred for identification by Dr. Y. Robin of the Institut Pasteur, Dakar. The virus is called Kedougou virus and was previously reported from YARU to be a probably new flavivirus. Three attempts to produce a hemagglutinating antigen from mouse brain by sucrose-acetone extraction were negative. Studies were done therefore by CF test. The antigen reacted with group B

Table 9

Identification of strain SA An-24630
Complement-fixation test

Antigen	Serum SA AN-24630
SA AN-24630	*1:256 or higher
Kunjin	1:256 or higher
West Nile	1:256 or higher
Alfuy	1:256
Kokobera	1:128
Koutango	1:64
Usutu	1:64

*Titer of serum. In addition, the serum gave a titer of 1:32 against 6 other group B antigens; 1:16 against 8; and 1:8 or lower against 27.

Table 10

Identification of strain SA An-24630
Complement-fixation test

Antigen	Serum	
	SA An-24630	West Nile
SA An-24630	256/64	256/128
West Nile	256/64	256/>128

Reciprocal of serum titer/reciprocal of antigen titer.

Table 11

HI reactions of SA Ar 19786 virus

Antigens	Antibodies					
	SA Ar 19786	UgS	YF	USU	D-1	D-2
SA Ar 19786	320	160	40	80	40	40
UgS	160	80	80	1280	160	160
yellow fever	40	80	160	160	320	160
Usutu	0	10	0	160	40	80
DEN-1	0	0	0	40	320	40
DEN-2	0	0	0	40	40	40

Table 12

CF reactions of NC Ar 250 virus[a]

Mouse Ascitic Fluids

Antigens	NC Ar 250	DEN-1	DEN-2	DEN-4
NC Ar 250	256/4[b]	32/16	4/16	32/16
DEN-1	64/16	16/16		
DEN-2	32/16		16/16	
DEN-4	512/64			32/64

[a]Kunjin, West Nile, and Zika antigens were negative against NC Ar 250 antibody 1:4.
DEN-3, Kokobera, Sepik, JE, MVE, and Tembusu antibody 1:4 were negative against NC Ar 250 antigen.

[b]Reciprocal of antibody titer/reciprocal of antigen titer.

grouping ascitic fluid, Ntaya (1:8), Usutu (1:32), West Nile (1:4), Zika (1:4), Spondweni (1:4), and dengue 1 (1:4). There was no reaction with Bouboui, yellow fever, Uganda S, Dengue 2, Dakar bat, Wesselsbron, or Banzi sera. Further, antigens of West Nile, Ntaya, dengue 1, and Usutu failed to react by CF with Dak Ar D 14701 ascitic fluid (homologous 128/256). The same homologous fluid did not inhibit Tembusu and Israel turkey meningoencephalitis HA antigens. Kedougou virus thus appears to be a new group B virus.

Japanese encephalitis virus (JE) virus from mosquitoes of Indonesia and relation of isolation frequency to vector density (J. Olson). The first isolation of JE from Indonesia was made from Culex tritaeniorhynchus collected in Kapuk, an area near Jakarta where pigs are raised (P.F.D. Van Peenen et al., 1974, Milit. Med., 139: 821-823). Additional studies on the ecology of JE in this focus showed that swine were amplifying hosts of the virus and the Cx. tritaeniorhynchus served as the principal vector species (P.F.D. Van Peenen et al., 1975, Trans. Roy Soc. Trop. Med. Hyg. 69: 477-479). The Virology and Entomology Departments of the U.S. Naval Medical Research Unit No. 2, Jakarta Detachment (NAMRU-2) began a study in October 1978 designed to test the hypothesis that JE activity was associated with vector density.

Mosquitoes were collected in 3 CDC light traps on each of 2 nights per month separated by 2-week intervals from October 1978 through May 1980. All blood fed female mosquitoes were held for 2 days to permit digestion of their blood meals, chilled, identified and separated by species into pools of approximately 50 each, and stored at -60°C. Each pool was thawed and triturated in phosphate buffered saline with bovine albumin added (pH 7.8 in a Ten Broeck grinder, centrifuged at 2113 xG for 20 minutes and the supernatant fluid stored at -60 °C until virus isolations were attempted.

Each triturated pool with antibiotics added was inoculated into tubes which contained a monolayer of green monkey kidney (Vero) and baby hamster kidney (BHK21) cells, respectively. Cells were observed daily for evidence of cytopathic effect (CPE) and negative blind passaged after 10 days. After 14 days subsequent to passage, specimens which failed to cause CPE were considered negative and discarded.

A total of 22 viruses were recovered from the 18,159 female Cx. tritaeniorhynchus mosquitoes in 366 pools. Six of these isolates have been identified as JE in a one-way microneutralization (Micro-Nt) test as described by T.G. Ksiazek and Liu (S.E. Asian J. Trop. Med. Publ. Hlth., (in press). Ten of the remaining 20 strains were lyophilized after passage in suckling mouse brain (smb) or cell culture to increase their titer and sent to YARU for identification. All but one strain was recovered and grown to sufficient titer for identification. Three of the 9 strains were tested by micro Nt in Vero cells using JE hyperimmune mouse ascitic fluid (HMAF) and tentatively identified as JE. Those strains which were not neutralized by anti-JE HMAF will be identified with other strains recovered from mosquitoes of Indonesia. Confirmation of the isolates presumptively identified as JE will be done by cross neutralization tests including other viruses and their antisera as well as HMAF prepared with

each field isolate. Table 13 gives the current status of the strains isolated from the study area.

BUNYAVIRUS

UG MP - 15332 (M. Kalunda and A. Mukuye). The strain was isolated from Aedes africanus wild-caught in Uganda, it produces CPE in Vero and BHK-21 cells and propagates well in newborn but not in adult mice. A sucrose-acetone antigen prepared from mouse brain tissue failed to agglutinate goose erythrocytes. Placement of the strain in an antigenic group was done by IF test; spot-slides carrying infected BHK-21 cells were tested against dilution 1:4 of the polyvalent grouping ascitic fluids listed above (see, SA AN 24630); in addition, the slides were tested with an Australian-viruses group antiserum (08174-6) and antisera against other Ugandan strains (Ug Sf 38485 and similar ones). A strong positive reaction was observed only with the Bwamba grouping reagent.

An antiserum for UG MP 15332 was next tested against antigens for the 6 viruses used in the preparation of the Bwamba polyvalent reagent, with the result shown in Table 14; the serum reacted only with Pongola and Bwamba antigens, with a higher titer with the former.

An additional CF test, Table 15, and a cross-neutralization test, Table 16, seemed to indicate that UG MP 15332 is a strain of Pongola virus.

Aus CH 19546 (R.E.Shope). This virus was referred for study by Dr. R. Doherty of the Queensland Institute for Medical Research, Australia. The original studies in this laboratory failed to detect relationship to other arboviruses. However, Dr. Doherty informed us that CH 19546 virus reacted by CF with the group Turlock grouping antibody. This relationship was confirmed. Neutralization test in baby mice with Aus CH 19546 virus and homologous and Umbre antibody indicated identity or a very close relationship (CH 19546 ascitic fluid LNI, greater than 5.0; Umbre serum LNI, greater than 5.0).

NAIROVIRUS

BREST AR/T 101 (K. Obom and J. Casals). This strain was isolated from Ornithodoros maritimus ticks collected in Brittany (France) in 1978, by Dr. C. Chastel, Medical School, Brest; the strain was found to be closely related to Soldado and confirmation was requested. Identification studies were begun in 1979 and completed this year.

The result of a CF test clearly shows, Table 17, that AR/T101 is closely related to Soldado virus (strain Cal AR 908). The results of neutralization test by intracerebral route in mice, Table 18, while confirming this conclusion as far as they go, were unsatisfactory owing to the low titers of the viruses.

More tests comparing AR/T 101 with additional strains of Soldado virus are required in order to decide whether this strain differs sufficiently and consistently from Soldado virus to justify considering it

Table 13

Virus Strains isolated from female Culex tritaeniorhynchus mosquitoes, Kapuk, 1978-1980

		Isolate	
Original pool/#	JKT Ar. Log #	Collected	Identified
4023	-	Oct. 78	ND
4026	657	Oct. 78	JE
4114	745	Dec. 78	JE
4157	788	Jan 79	JE
4161	792	Jan. 79	JE
4180	811	Jan. 79	JE
4244	1110	Feb. 79	JE
4889	1729	Apr. 79	
4909	1749	Apr. 79	
4914	1754	Apr. 79	
5272	1977	Mar. 79	
6262	2159	Aug. 79	
6307	2204	Oct. 79	
6315	2212	Oct. 79	
6322	2219	Oct. 79	
6339	-	Nov. 79	
6365	2329	Nov. 79	
6388	-	Nov. 79	
6398	2362	Nov. 79	
6399	2363	Nov. 79	
6556	-	Dec. 79	
6557	-	Dec. 79	
6673	-	Jan. 80	
6979	-	May 80	

*Tentatively identified.
ND=Not done.

Table 14

Identification of strain UG MP-15332
complement-fixation test

Antigen	Serum UG MP-15332
UG MP-15332	128/$\geq$16*
Bwamba	64/$\geq$16
Eretmapodites	0
Kamese	0
Mossuril	0
Nyando	0
Pongola	128/$\geq$16

*Reciprocal of serum titer/reciprocal of antigen titer; first dilution of serum, 1:8.

Table 15

Identification of strain UG MP-15332
complement-fixation test

Antigen	Serum UG MP-15332	Pongola
UG MP-15532	128/256*	32/128
Pongola	128/512	32/256

*Reciprocal of serum titer/reciprocal of antigen titer.

Table 16

Identification of strain UG MP-15332
Neutralization test by IC route in mice

Serum	Virus: Pongola IC LD_{50}	Pongola Log NI	Ug Mp-15332 IC LD_{50}	Ug Mp-15332 Log NI
Pongola	$10^{-3.9}$	2.4	$10^{-3.2}$	3.4
UG MP-15332	$10^{-5.0}$	1.3	$10^{-5.0}$	1.6
Control	$10^{-6.3}$		$10^{-6.6}$	

NI, Neutralization index.

Table 17

Identification of strain Brest Ar/T101
Complement-fixation test

Antigen	Serum: AR/T101	Soldado
Ar/T101	256/64	32/32
Soldado	64/32	128/64
Farallon	32/10	4/8
Hughes	TRACE	8/16
Punta Salinas	16/16	4/8
Zirqa	8/16	8/8

Reciprocal of serum titer/reciprocal of antigen titer.

as a new virus in the Hughes group; at the moment it seems adequate to consider BREST AR/T 101 a subtype of Soldado virus.

BREST AR/T 234. (J. Casals). Strain isolated from O. (A.) maritimus from Essaouira island, off Mogador, Morocco, in 1979; submitted by Dr. C. Chastel, who found the strain close to Soldado virus. The result of a CF test, Table 19, established that by this test the strain is indeed close to Soldado but distinct. It determines the relationship between this strain and the preceeding one (Brest AR/T 101).

Omo. (J. Casals and R.E. Shope). Omo virus was isolated from the spleen of Mastomys spp. caught at Ethiopia, in the Omo River Valley, in 1971, and submitted by Dr. P.Ardoin, Pasteur Institute, Paris. Screening by CF test had shown (YARU Annual Report, 1973) that the virus was related to Qalyub virus. Continuation of these studies by CF have given the results shown in Table 20, which indicate that the two viruses, while closely related, appear to be distinguishable by this test. Comparison of the two agents by plaque reduction tests is planned.

ORBIVIRUS

MEUZ STRAIN (J. Casals). This agent was isolated from the blood of a febrile patient in Amsterdam, the Netherlands, by Dr. H.A.E. van Tongeren, of that city. The patient had traveled in the Rocky Mountains area during the late part of May, 1979, and developed a febrile illness with onset of June 2. This virus was isolated from blood taken on June 8; Dr. Tongeren suspected Colorado tick fever and submitted the virus for identification.

The result of a CF test, Table 21, with a hyperimmune mouse serum for the Meuz strain and a reference CTF immune serum showed that the new strain was similar to two CTF virus strains, 65-68 and Condon. Final identification was done by intracerebral mouse neutralization test with the result shown in Table 22. It appears that Meuz is a strain of CTF.

Fr Brest/Ar/T222 (A.J.Main). This strain originally isolated from a pool of 10 adult female Ornithodoros maritimus collected from herring gull nests on Essaouira Island, near Mogador, Morocco during June 1979, was submitted for identification by Dr. C. Chastel of Brest, France. Complement-fixation and plaque-reduction neutralization tests indicate that it is a member of the Chenuda complex of the Kemerovo serogroup close to Chenuda and Baku, Table 23.

BeAr 236191 and BeAr 295042 (D.L.Knudson). These two viruses from Culex mosquitoes were referred by Dr. F. Pinheiro, Belem, Brazil with information that they reacted with NIH polyvalent Palyam-Corriparta grouping fluid. CF tests at YARU confirmed the reactivity with the grouping fluid and demonstrated that the 2 Brazilian isolates belong in the Corriparta serogroup, Table 24.

RHABDOVIRUS

Natal bat virus (G.H.Tignor and J. Crick). A virus isolated from bats

Table 18

Identification of strain BREST AR/T101
Neutralization test by IC route in mice

Serum	Virus			
	Brest AC/T101		Soldado	
	$ICLD_{50}$	log NI	$ICLD_{50}$	log NI
Ar/T101	$10^{-1.8}$	2.0	$10^{-1.6}$	0.8
Soldado	$10^{-2.4}$	1.4	$10^{-1.5}$	0.9
Control	$10^{-3.8}$		$10^{-2.4}$	

NI, neutralization index

Table 19

Identification of strain Brest Ar/T234
Complement-fixation test

Antigen	Serum	
	Ar/T234	Soldado
Brest Ar/T234	256/>128	32/128
Soldado	128/32	256/64
Farallon	32/32	16/16

Reciprocal of serum titer/reciprocal of antigen titer.

Table 20

Identification of Omo virus
Complement-fixation test

Antigen	Serum		
	Omo	Qalyub	Bandia
Omo	256/>256	128/256	16/128
Qalyub	128/64	256/128	32/64
Bandia	32/128	64/128	256/>256

Reciprocal of serum titer/reciprocal of antigen titer.

Table 21

Identification of strain MEUZ
Complement-fixation test

Antigen	Serum	
	MEUZ	CTF65-68
MEUZ	128/256	128/256
CTF, strain 65-68	64/256	128/512
CTF, strain Condon	64/256	128/256

Reciprocal of serum titer/reciprocal of antigen titer.

Table 22

Identification of strain MEUZ
Neutralization test by IC route in mice

Serum, mouse	Virus			
	MEUZ		CTF, Condon	
	$ICLD_{50}$	NI	$ICLD_{50}$	NI
MEUZ	$10^{-1.5}$	4.2	$10^{-3.9}$	4.4
CTF, strain 65-68	$10^{-1.5}$	4.2	$10^{-3.5}$	4.8
Control	$10^{-5.7}$		$10^{-8.3}$	

NI, neutralization index.

Table 23

Serological identification of strain Brest/Ar/T222

	Brest/Ar/T222			
	Antigen		Ascitic Fluid/Serum	
	CF	NT	CF	NT
Chenuda	32/128*	10/640	128/128	<10/320
Baku	128/256		64/128	
Mono Lake	32/256	<10/640	32/128	<10/320
Huacho	<8/128	10/ -	<8/128	-
Kemerovo	<8/256	<10/ -	<8/128	-
Tribec	16/64	<10/ -	<8/128	-
Lipovnik	<8/32	-	<8/128	-
Fin V808	<8/128	<10/ -	<8/128	-
Fin V962	<8/256	<10/ -	<8/128	-
Mykines	<8/256	<10/ -	<8/128	-
Great Island	<8/64	<10/ -	<8/128	-
Bauline	<8/8	<10/ -	<8/128	-
Cape Wrath	<8/16	<10/ -	<8/128	-
Yaquina Head	<8/128	<10/ -	<8/128	-
Okhotskiy	<8/128	<10/ -	<8/128	-
Nugget	64/8	<10/ -	<8/128	-
Wad Medani	<8/16	<10/ -	<8/128	-

*Homologous/heterologous titers

in South Africa was sent by Dr. C. D. Meredith of the Institute for Virology, Sandringham. In June, 1980, an outbreak of bat rabies in Durban, Natal concurrent with an epizootic of dog rabies was noted. The Natal bat virus was initially tested by CF with rabies virus at YARU and shown to differ. Subsequent CF tests indicate that Natal bat virus is identical or closely-related to Lagos bat virus Table 25. This is the first isolate of Lagos bat virus in South Africa.

UNCLASSIFIED

Ug Z-52969. (M. Kalunda and A. Mukuye). The strain was isolated from the viscera of a fruit bat, Rousetus aegyptiacus, in Uganda; a second, similar strain was isolated from another bat of the same species at about the same time. Furthermore, shortly after the virus was introduced in the Entebbe laboratory, four additional strains of what appeared to be the same agent were isolated from laboratory workers who had developed a mild to severe illness.

Work in this laboratory showed that the virus propagated in Vero and BHK-21 cells in the absence of CPE; the agent killed newborn and adult mice. IF tests with polyvalent grouping immune ascitic fluids and with a hyperimmune Lassa virus antiserum gave negative results.

A hyperimmune mouse serum for UG Z-52969 in serial two-fold dilutions beginning at 1:4 was tested by CF test against 79 different antigens; and 22 antisera for viruses not included in the polyvalent grouping reagents, Table 26, were similarly tested against an antigen for this strain. The only cross-reaction noticed in these tests was with Yogue virus. Additional CF tests clearly established the relationship between Ug Z-52969 and Yogue, Table 27, indicating that the two viruses constitute a new antigenic group. Investigation of the relationship between these two agents is continuing, by means of the neutralization test.

By electron microscopy, Vero and BHK-21 cells infected with this agent showed viral particles similar to those of Bunyaviruses.

Mazoe virus: Serological relationship with Ippy virus. (J. Casals). In last year's annual report was given the result of a CF test that seemed to indicate that these 2 viruses were related; however, some doubts were at the time expressed concerning the validity of the observed cross-reaction. Subsequent CF tests using the same sample of Ippy immune serum against normal tissue and unrelated antigens have shown that the reaction against Mazoe antigen given by this particular Ippy antiserum was non-specific. Cross-neutralization tests by the intracerebral route in mice with Mazoe and Ippy viruses and their antisera have given the results illustrated in Table 28; these results show no relationship between the two viruses.

Can 50-51 (A.J.Main). This strain, originally recovered from Dermacentor variabilis in Canada, was submitted for identification by Dr. Harvey Artsob of the National Arbovirus Reference Service Toronto, Ontario. A sucrose-acetone extracted antigen was tested by complement-fixation against the following specific and polyvalent ascitic fluids with

Table 24. Brazilian isolates: Complement-fixation tests with Corriparta serogroup viruses.

Antigen	Mouse As F			
	BeAr 295042	BeAr 236191	Acado (EthAr 1846-64)	Corriparta
BeAr 295042	4/4	8/8	64/32	4/8
BeAr 236191	0	8/8	32/32	4/8
Acado	4/4	8/8	256/128	4/16
Corriparta	0	0	32/8	4/8

Table 25

CF Identification of the Natal bat strain of Lagos bat virus[a]

			Antibody		
Antigen	Natal bat	Lagos bat	Rabies (CVS)	Duvenhage	Mokola
Natal bat	512/1024[b]	512/512	32/64	8/8	256/128
Lagos bat	512/256	512/512		32/32	128/128
Rabies (CVS)	8/8		256/ 256		
Duvenhage	32/32	32/8		32/64	
Mokola	128/64	64/64			512/256

[a]Composite of 3 CF tests.
[b]Reciprocal of antibody titer/reciprocal of antigen titer.

Table 26

Viruses Tested by CF Against UGZ 52969

Antigens

Abu Mina	Colorado tick	Kowanyama	Silverwater
Acado	Corriparta	Kwatta	Simbu
Acara	Cotia	Lagos bat	Soldado
Akabane	Cowbone Ridge	La Joya	Sororoca
Amapari	Dengue 2	Lanjan	Sud An 754-61
Anhanga	Dera Ghazi Khan	Lebombo	Sud Ar 1169-64
Anopheles A	Dhori	Le Dantec	Tacaiuma
Anopheles B	Dugbe	Lone Star	Tamiami
Apeu	EEE	Lukuni	Tataguine
Arkonam	EgAn 1398-61	LCM	Tembe
Aruac	EgAn 1825-61	Manzanilla	Tensaw
Arumowat	EHD-NJ	Mapputta	Tete
Aus MRM 4059	Eretmapodites 147	Marco	Thimiri
Aus Ar 8659	Eubenangee	Matariya	Thogoto
Aus C 12048	Flanders	Matucare	Thottapalayam
Aus CH 9935	Gamboa	Melao	Timbo
Aus MK 6357	Germiston	Minititlan	Triniti
Aus MRM 10434	Gossas	Minnal	Trivittatus
Bahig	Grand Arbaud	Mirim	Tsuruse
Bakau	Guajara	MML	Turlock
Bandia	Guama	Modoc	Ug MP 359
Bangoran	Guaroa	Mokola	Umbre
Bangui	Hazara	Mossuril	Upolu
Bauline	Herpes	Mt Elgon bat	Utinga
BeAn 67949	Hilo	Nariva	Uukuniemi
BeAn 84381	Huacho	Navarro	Vaccinia
BeAn 100049	Hughes	NDV	VEE
BeAn 141106	I 6235	Nkolbisson	VS-New Jersey
BeAn 174214	I 61-2629	Nola	VS-Indiana
BeAn 177325	IbAn 8341	Nyamanini	Wad Medani
Bertioga	IbAn 20433	Nyando	Wanowrie
Bhanja	IbAn 28946	Okola	WEE
Bluetongue	IbAn 38918	Olifantsvlei	Witwatersrand
Bobia	Ichampadi	Oropouche	Wongal
Bocas	Ieri	Oyo	Wyeomyia
Boteke	Ilesha	Pacora	Yaba-1
Botambi	Irituia	Pacui	Yata
Bujaru	Itaporanga	Palyam	Yellow fever
Bushbush	Itaqui	Pata	Yogue
Buttonwillow	Joinjakaka	Patois	Caraparu
Bwamba	Jos	Pichinde	Makindu
California	Junin	Powassan	Ug Sg 37317/9
Candiru	Jurona	Punta Toro	EgArT 904
Capim	Kaeng Khoi	Qalyub	Indo 1039
FG Ar 564	Kairi	Quaranfil	USSR Cq 13
Chaco	Kammavanpettai	Rabies	Ken T 83
Chagres	Kannamangalam	Reovirus	Tettnang
Chandipura	Karimabad	Rio Bravo	EgArT 1147

Table 26 (continued)

Changuinola	Kemerovo	Salehabad	Mal P 350
Charleville	Kern Canyon	SF-Naples	EgArT 427
Chenuda	Keterah	SF-Sicilian	Han 70-3-28
CHF-Congo	Klamath	Sathuperi	P 72-4R
CoAr 1279	Koongol	Sawgrass	BFN 3187
Cocal	Kotonkan	Sembalam	Oita 296

Abu Hammad	Punta Salinas
Eyach	Wallal
Kao Shuan	Warrego
Mitchell River	Zaliv Terpeniya
Pretoria	Zegla
Mal P 350	Zirqa
Almpiwar	BeAn 116382
Belmont	BeAn 123048
Chobar Gorge	BeAn 35646
Japanaut	BeAn 41067
Ug MP 359	BeAn 185559
Zinga	I 47
EgAn 5056	IbAn 17854
Eth An 3530	M 1146
Eth An 4759	Ph Ar 281
BeAn 327600	Piry
BeAn 20076	PH 814
BeAn 109303	TR 51121
	UG-SG-37317

Antisera

Aride	Ken T 100
Barur	Manawa
Boraceia	Malakal
Bov.Eph.Fever	N.S.D.
Bimbo	Nodamura
Clo-Mor	Pahayokee
Gomoka	Sakhalin
Ippy	Shark River
Kolongo	Swan P 25
Ken Makindu	Tanga
Ken T 99	Venkatapuram

Table 27

Relationship between UGZ-52969 and Yogue viruses
Complement-fixation test

Antigen	Serum			
	UGZ-52969		Yogue	
	#1	#2	#1	#2
UGZ-52969	128/32	256/16	8/4	32/8
Yogue	64/128	64/128	256/256	512/128

Reciprocal of serum titer/reciprocal of antigen titer.

Table 28

Absence of antigenic relationship between Ippy and
Mazoe viruses by IC mouse neutralization tes

Serum	Virus			
	Mazoe		Ippy	
	$ICLD_{50}$	Log NI	$ICLD_{50}$	Log NI
Mazoe	$10^{-5.3}$	2.5	$10^{-6.2}$	0.2
Ippy	$10^{-7.6}$	0.2	$10^{-3.7}$	2.7
Control	$10^{-7.8}$		$10^{-6.4}$	

NI, Neutralization index.

negative results: eastern equine encephalomyelitis, western equine encephalomyelitis, Highlands J, Tyuleniy, Modoc, Powassan, Cache Valley, California encephalitis, Jamestown Canyon, snowshoe hare, trivittatus, La Crosse, Keystone, Great Island, Connecticut, Colorado tick fever, Chobar Gorge, Flanders, mouse hepatitis, herpes, Microtus, Clethrionomys gapperi, Bahig, Tete, Matruh, Matariya, Burg el Arab, Eg 1398, Koongol, Wongal, Bakau, Ketapang, Mapputta, Trubanaman, MK7532, Nyamanini, Uukuniemi, Grand Arbaud, Thogoto, Hughes, Sawgrass, Matucare, Lone star, Soldado, Hart Park, Kern Canyon, Klamath, Mount Elgon bat, bluetongue, epizootic hemorrhagic disease of deer, IbAr 22619, Changuinola, Irituia, Navarro, Triniti, Aruac, Pacora, Upolu, Dera Ghazi Khan, Wanowrie, Dhori, Okola, Olifantsvlei, Witwatersrand, Tataguine, Dak-1569, Quaranfil, Bandia, Kaisodi, Johnston Atoll, Lanjan, Qalyub, Silverwater, Congo-Crimean hemorrhagic fever, Ganjam, Dugbe, Bhanja, rabies, lymphocytic choriomeningitis, Newcastle disease, vaccinia, group B, group Sakhalin, group Uukuniemi, group vesicular stomatitis, and group Kemerovo. Can 50-51 virus appears to be a new, ungrouped tick-borne virus.

I 77-236-17 (R.E.Shope). This virus isolated from Ornithodoros piroformis ticks, was referred for identification by Dr. K. Pavri, Pune, India. A sucrose-acetone extracted mouse brain antigen did not react by CF test with mouse ascitic fluids of group A, group B, polyvalent Australia, polyvalent Patois, group Simbu, group Guama, NIH polyvalent 2,3,4,12, polyvalent Palyam-Changuinola-Corriparta, mouse hepatitis, polyvalent Rabies-LCM-vaccinia-herpes-NDV, group Bunyamwera, and group Phlebotomus fever. The homologous reaction was 64/40. Additional tests are planned.

Identification of virus strains recovered from mosquitoes and bats in Indonesia (J. Olson). The virology and entomology Departments of the U. S. Naval Medical Research Unit No. 2, Jakarta Detachment have made collections of arthropods and small mammals from many locations in Indonesia in order to determine the geographic distribution and host ranges of arthropod-borne viruses in Indonesia. On two separate occasions arthropods were collected from areas in Sumatra where families from Java have been relocated by the Government. These sites are ideal for the transmission of arthropod-borne viruses since they bring man in close contact with forest dwelling arthropods. Each area was originally claimed from the forest and now constitutes an enclave surrounded by the forest. Many inhabitants derive all or a portion of their income from wood cutting, rubber tapping or other occupations for which they must go into the forest daily.

To date only 2 strains of virus have been recovered from the pools of mosquitoes collected in these areas. Two strains of JE have been identified from Cx. whitemories and Cx. gelidus from South Sumatra. Two not yet identified viruses have been recovered from 2 species of bats, Eonycteris spelaea and Cynopterus brachyotis captured in West Sumatra.

In 1978 a serologic study of human and animal populations of Lombok, the Lesser Sundase, showed that neutralizing (NT) antibodies to several arthropod-borne viruses were prevalent. More than 30% of humans tested had hemagglutination-inhibiting (HI) antibody to flaviviruses. Of those

persons with HI antibody, 13% had NT antibody to Zika virus, 13% to dengue type 2 (DEN-2) and there were individuals with NT antibody to Tembusu (TMU) Kunjin (KUN) and Sepik (SEP) viruses. Domestic animals showed evidence of infection with JE, Murray Valley encephalitis (MVE), KUN, SEP, and Batai (BAT) viruses.

In March 1979, a following study was completed after the annual rains had begun. Collections were made of 144,701 mosquitoes and more than 48,000 Culicoides spp. Efforts were concentrated around animal stables and in villages where prevalences of antibodies were high. From the 1,022 mosquito pools, 13 virus strains were recovered from 8 species. No isolations were made from Culicoides spp. Table 29 shows the species from which viruses were recovered.

III. SEROLOGIC SURVEYS

Human surveys were carried out by means of the immunofluorescence test (IF) using spot-slides prepared and inactivated in other laboratories or at YARU; all sera screened at dilution 1:4.

Ghana (J. Casals). Sera were collected in June 1975, and deposited in the WHO serum center, Yale. Thus far, 146 sera have been tested with polyvalent slides containing a mixture of cells infected with Ebola, Lassa and Marburg viruses (ELM); positive sera were subsequently tested with the monovalent slides. Ten sera had antibodies against Ebola virus only; in addition one reacted with Ebola at dilution 1:16 and with Marburg at dilution 1:4. No antibodies were found against Lassa virus. This is the first report of Ebola antibodies in Ghana; furthermore, it is to be noted that these sera were collected in June 1975, one year before the first recognized outbreak of Ebola disease.

Cameroon. Anti-Ebola antibodies have been detected in 5 of 41 sera from native employees at a missionary hospital in Garoua-Bolai.

Liberia. In a continuing surveillance for Lassa virus antibodies centered in missionary hospitals in Liberia, conducted in association with Dr. J. D. Frame, Columbia University Presbyterian Medical Center, 196 human sera have been tested since January 1, 1980; 16 of them were positive.

Survey of Sinai Bedouin sera for antibody to Rift Valley fever (R.E.Shope). Thirty-three sera of Bedouins indigenous to the Sinai were submitted by Dr. T. Schwartz, Department of Epidemiology, Israeli Ministry of Health. Tests in Jerusalem had shown antibody to RVF. These results were readily confirmed by HI test using a mouse liver antigen supplid by USAMRIID, Table 30. Eight of 33 sera tested against 8 antigen units were positive. Three additional sera showed partial inhibition. One of these three was positive when screened at 1:10 dilution with Saint-Floris antigen as were 4 of RVF HI reactors. RVF and Saint-Floris reactions correlated well as would be expected since both are in the Phlebotomus fever group. The sera were also screened at 1:10 with Sindbis and West Nile antigens. Three reacted with Sindbis and 10 with West Nile. There

Table 29

Virus Strains Isolated from Mosquitoes and Bats
Indonesia, 1978-1980

Isolate (JKT Ar. Log#)	Species	Collection Location	Collection Month/Year	Identification
451	Culex tritaeniorhynchus	Lombok	May 78	JE
813	Culex fuscocephala	Kapuk	Jan 79	JE
974	Culex gelidus	Kapuk	Jan 79	JE
1105	Culex gelidus	Kapuk	Feb 79	JE
1431	Culex tritaeniorhynchus	Lombok	Mar 79	
1437	Culex vishnui	Lombok	Mar 79	
1438	Culex vishnui	Lombok	Mar 79	
1545	Anopheles vagus	Lombok	Mar 79	
1574	Anopheles tesselatus	Lombok	Mar 79	
1657	Aedes lineatopennis	Lombok	Mar 79	
1661	Culex whitmorei	Lombok	Mar 79	
1664	Culex tritaeniorhynchus	Lombok	Mar 79	
1724	Culex tritaeniorhynchus	Lombok	Mar 79	
1989	Culex vishnui	Kapuk	May 79	
2046	Culex tritaeniorhynchus	Lombok	Mar 79	
2065	Aedes vexans	Lombok	Mar 79	
2254	Anopheles annularis	Lombok	Mar 79	
2267	Anopheles vagus	Lombok	Mar 79	
2527	Rousettus amplicaudatus	Lombok	Mar 79	
2774	Eonycteris spelaea	W.Sumatra	Apr 79	
2816	Cynopterus brachyotis	W.Sumatra	May 79	

Table 30

HI Reactions of Sinai Bedouin Sera to RVF and other Antigens

Serum no.	RVF	St. Flor.	Sind	West Nile	Serum no.	RVF	St. Flor.	Sind	West Nile
1	0*	0	0	+	18	0	0	0	0
2	0*	0	+	0	19	0	0	0	+
3	0	0	0	0	20	0*	+	0	0
4	0	0	0	0	21	0	0	0	0
5	0	0	0	0	22	0	0	0	0
6	0	0	0	0	23	320	+	0	0
7	0	0	0	0	24	0	0	+	0
8	0	0	0	0	25	10	+	0	0
9	0	0	)	0	26	0	0	0	0
10	20	0*	0	0	27	0	0	0	0
11	40	+	0	0	28	10	0*	0	+
12	0	0	0	0	29	0	0	0	+
13	40	+	0	+	30	0	0	0	+
14	40	0*	0	+	31	0	0	0	0
15	0	0	0	+	32	10+	0*	0	+
16	0	0	+	+	33	0	0	0	0
17	0	0	0	0					

*Partial reaction.

was no direct correlation among RVF, Sindbis, and West Nile reactors.

These results appear to be evidence for RVF in the Sinai area although interpretation will depend on travel history of the Bedouins and on further tests to determine if these reactions are specific for RVF or are caused by exposure to other Phlebotomus fever group viruses.

Survey for antibodies to arthropod-borne viruses in Sudan (J. Meegan, D. Winograd, and T. Bucci (NAMRU-3)). It is likely the many arthropod-borne viruses which circulate in northern and southern Africa are disease problems in Sudan. Few studies have been undertaken in this geo-politically important country. During 1979, 1980, and 1981 over 1200 sera were collected from military recruits in Sudan. These represent collections from all areas of Sudan (age, birthplace, and district of residence are available for all). This survey is the first phase of a long-term study to determine the impact of arthropod-borne viruses on human beings and animals in Sudan. In addition, since Sudan may act as a tunnel for the movement of viral disease from sub-Saharan Africa to Egypt and beyond, survey for other arboviruses may give clues as to what diseases to be alert for.

To date, 708 sera have been screened by the indirect immunofluorescent (IF) test. Micro-neutralization (N) tests are in progress to validate the preliminary IF results. Table 31 presents our initial IF results. Antigens employed in these tests were viruses implicated as human pathogens in equatorial, northern, and southern Africa.

Many of the viruses to which antibodies were detected have not been thoroughly investigated. For example, the high prevalence of antibodies to Tataguine and Zinga/Bangui was unexpected, and if confirmed by N tests, would indicate these viruses are widespread in equatorial Africa. Preliminary N tests have validated that the antibodies to SAF, GOR, and Gabek Forest (GF) are specific, and thus represent human infections with these agents. IF cross-reactions and nonspecific positive fluorescence most likely make the prevalence rates in Table 31 higher than the true rate determined by N tests.

In conjunction with an ongoing survey for antibodies to Lassa, Marburg, and Ebola viruses in equatorial Africa (see other sections this report), sera from southern Sudan were screened for these antibodies. Sixty-nine sera have been tested to date; 14 were positive with the trivalent antigen slide. Four of these positive sera were tested with monovalent slides; three were positive only for Ebola, and one gave questionable results. Further testing is in progress to characterize the positive results and to evaluate sera from other geographic areas within Sudan.

IV. DIAGNOSIS OF DISEASE

Korean hemorrhagic fever (J. Casals). The application of IF to the diagnosis of KHF was made possible by the propagation of the disease's

Table 31

Immunofluorescent tests of human sera from Sudan for antibodies to selected arthropod-borne viruses

Antigen	Number positive/Number tested
Polyvalent Alphavirus (CHIK/SIN/ONN)	30/708
Flavivirus (WN)	243/708
Bunyamwera	
BUN	47/708
GER	55/708
ILE	34/708
Bwamba	
BWA	15/708
California	
TAH	5/500
Phlebotomus	
SFS	121/708
SFN	20/708
AMT	11/708
SAF	29/708
GOR	22/708
GF	42/708
RVF	27/846*
Kemerovo	
KEM	10/708
WM	4/500
Nairovirus	
DVG	9/708
QRF	2/350
Thogoto	
THO	2/500
Simbu	
SHU	5/500
Nyando	
NDO	1/230
Ungrouped	
Bivalent Zinga/Banqui	43/708
Acado	0/150*
Malakal	0/150*
Polyvalent Ebola/Marburg/Lassa	14/69

*Results of CF tests.

etiological agent in cell cultures by H.W. Lee (1978). Spot-slides containing suspensions of a human lung carcimona cell line - A 549-infected with KHF virus were supplied by Dr. G. French, USAMRIID; control slides with uninfected cells were also supplied. Three sets of sera were tested with these slides in an attempt to determine their suitability as diagnostic antigens:

a. Fifteen convalescent sera from previous admissions to the Hospital of the Hubei Provincial Medical College, Wuchang, China, between 1975 and 1979, with the clinical diagnosis of epidemic hemorrhagic fever (Korean hemorrhagic fever, KHF); these sera were supplied by Dr. C.M. Hsiang, of that medical school.

b. Ten paired sera from patients admitted to 3 hospitals in South Korea in 1974, clinically diagnosed as KHF; sera supplied by Dr. K.H. Kim, National Institute of Health, Seoul, Korea.

c. Single sera from 10 U.S. Army personnel, with the clinical diagnosis of KHF, in 1967-68; sera supplied by Dr. N. Wiebenga, Chief, Epidemiology Branch, Department of Health, Hawaii.

The sera were tested by IF test only at dilution 1:4, with the exception of two that were titrated out.

Results: All the sera from group a gave positive reactions with readings rating 3 or 4, except one that was considered as questionable; the reactions with the control, uninfected slides were uniformly negative.

The results with sera in groups b and c are presented in Table 32. Positive reactions were given by all the patients except #KHF-5; this may have been a false clinical diagnosis. Acute sera taken as early as the 6th day from onset were strongly positive; titering out of two sera, J.H.L. and J.D.Y., resulted in high titers, 1:2048 and 1:512, respectively.

The results given by these 3 groups of patients simply confirm the observations by others and show that the IF test is, at this time, a reliable procedure as an aid to the diagnosis of KHF.

CCHF. A number of laboratory workers at this Unit have been involved in investigations with CCHF virus. In keeping with recent restrictions imposed on the use of this agent, all activities requiring the use of active, virulent virus have been discontinued. None of the individuals connected with work with this virus over a period of years had, at any time, an illness that could even remotely be considered CCHF. In order to determine whether subclinical or aberrant infections could be documented, sera from 7 persons who, at some time or other, participated in the work were tested by IF test against CCHF antigen on "spot-slides"; the sera were tested at dilution 1:4. The number of sera from each individual were: PB, 2; JC, 3; CM, 3; RS, 7; AS,1; GT, 2; and HW, 5. All sera gave negative reactions; it appears, therefore, that CCHF virus can be handled in the laboratory with safety.

Table 32

Diagnosis of Korean hemorrhagic fever, indirect immunofluorescence test

Patient identification	Serum			Result	
	Days from onset		Days from Hospital admission		
	Acute	Late		Acute	Late
KHF-1	8	15		3	4
KHF-2	6	13		3	4
KHF-3	6	16		3	2
KHF-4	7	21		4	3
KHF-5	6	35		0	0
KHF-6	13	35		3	3
KHF-7	12	34		3	4
KHF-8	7	38			4
KHF-9	11	42		4	4
KHF-10	14	45		4	4
D.L.			21		2
R.R.			28		4
J.H.L.			28		4 (1:2048)
S.K.K.			32		4
J.D.V.			28		4 (1:512)
M.C.			29		4
J.T.			30		4
L.G.			27		4
H.B.C.			26		3
J.K.L.			36		4

Sera tested at dilution 1:4; intensity of fluorescence in a 0 to 4 scale.

Identification of agents recovered from fever patients in Klaten, Central Java (J. Olson). There is serologic evidence that Zika causes fever among inpatients in Klaten. (Olson, et al., Trans. Roy. Soc. Trop. Med. Hyg., in press).

A following study in the same hospital was designed to identify the relative importance of bacteria, rickettsia and viruses as etiologic agents among inpatients. The study began in December 1978, and continued through January 1980. A total of 12 agents was recovered in Vero or BHK-21 cells from acute phase sera collected from febrile patients. Table 33 summarizes the signs, symptoms and test results of the patients from whom an isolate was recovered.

All isolates were passaged in mice or cell cultures to increase their titer and then lyophilized and sent to YARU for identification. All but one isolate (JKT Ar.Log#2555) has been recovered at YARU and titrated to contain at least $10^{4.0}TCID_{50}/0.025$ ml. To date, unidentified agents have been set upon spot-slides for grouping by immunofluorescence test.

Fever Studies in Indonesia (J. Olson). An integral part of the arbovirus studies in Indonesia has been the performance of short term and longitudinal studies of patient populations who seek treatment. A variety of populations has been studied longitudinally and includes pediatric inpatients with encephalitis from Java, pediatric inpatients from Sulawesi with febrile illnesses, febrile inpatients of all ages from Bali, urban and rural outpatients with fever from Bali and urban inpatients of all ages with febrile illnesses. Short term fever studies of outpatients who seek medical care in government clinics were also completed in Lombok and Sumatra. In each case our protocols stressed both serologic examination and isolation of arthropod-borne viruses. All isolation attempts were completed in Indonesia and discussions of our attempts to characterize those viruses not identified as dengue are discussed in another section of this report.

Serologic examination of patients stressed the collection of acute and convalescent sera and careful completion of patient records and check list of signs and symptoms. Table 34 shows the distribution of patients, using the various studies and the serologic tests already completed for each group. Our plan is to test paired sera for neutralizing antibodies and determine the relative importance of arthropod-borne viruses as causes of illness in the samples.

We have already compiled a list of viruses known to be present in Southeast Asia and the Western Pacific, Table 35. Working stocks of these viruses are being grown to yield $TCID_{50}$ of $10^{-4.0}\log_{10}/0.025$ ml. for use in a microneutralization test (Ksiazek and Liu, in press). Table 36 shows the status of our efforts. Each reference strain to be used in our micro-NT test will be tested with patient sera and reference hyperimmune mouse ascitic fluids (HMAF).

Dengue virus is highly endemic throughout most of Indonesia and will

Table 33

Klaten inpatients from whom agents were recovered from acute phase sera

Study #	JKT Ar. log #	Age	Sex	Date of onset
4003	2541	20	F	13 Feb.79

Laboratory: no pathogens isolated, serology not done

Summary: 37.5°C, headache, myalgia, cough, diarrhea, abdominal pain.

4006	2543	7	M	10 Feb. 79

Laboratory: *Salmonella typhi* recovered, serology not done.

Summary: 37.5°C, chills, pharyngitis, rhinorrhea, weakness.

4020	2553	12	F	17 Feb. 79

Laboratory: No pathogens isolated; serology not done.

Summary: 39.6°C, headache, nausea, vomiting, constipation, abdominal pain, anemia.

4023	2555	25	M	18 Feb. 79

Laboratory: *Salmonella typhi* recovered, 1:1280 IFA antibody titer to *Rickettsia typhi*

Summary: 40.0°C, headache, abdominal pain.

4024	2556	11	M	19 Feb. 79

Laboratory: No pathogen isolated, serology not done.

Summary: 38.3°C, headache, nausea, constipation, abdominal pain.

4025	2557	18	F	18 Feb. 79

Laboratory: *Salmonella typhi* recovered, serology negative

Summary: 39.0°C, headache, apathy, backache, myalgia, nausea, vomiting, diarrhea, abdominal pain.

Table 33 (continued)

Study #	JKT Ar.log #	Age	Sex	Date of onset
4033	2564	14	F	15 Feb 79
Laboratory:	Salmonella typhi recovered, serology not done			
Summary:	39.2°C, somnolent, apathy, constipation, abdominal pain, shock, death.			
4058	2573	18	M	6 May 79
Laboratory:	not reported, serology not done			
Summary:	38.2°C, headache, myalgia, chills, constipation.			
4184	2577	NA*	NA	NA
Laboratory:	NA			
Summary:	NA			
4187	2578	19	M	28 JUL 79
Laboratory:	no pathogens isolated, serology negative			
Summary:	39.0°C, headache.			
4224	2591	40	F	27 Sept.79
Laboratory:	Salmonella typhi recovered, serology negative			
Summary:	39.0°C, headache, hematemesis, nausea, vomiting, constipation, abdominal pain, edema, cirrhosis, hepatitis			
4302	2966	17	F	7 JAN 80
Laboratory:	no report, serologic response to B influenza			
Summary:	38.7°C, headache, backache, cough, pharyngitis, chest pain, nausea, abdominal pain.			

*NA=not available.

Table 34
Fever Studies in Indonesia

Location	Study type	Population source	No. of patients	Positive serology
Sumatra	short term fever study	outpatients attending Clinic (all ages)	75	3 flavivirus 2 alphavirus 5 influenza 4 rickettsiae
Lombok	short term fever study	outpatients attending Clinic (all ages)	47	5 influenza
Jakarta, Java	longitudinal encephalitis	inpatients (pediatric)	115	?flavivirus 1 mumps
Klaten, Java	longitudinal fever study	inpatients (all ages)	120	7 flavivirus 5 alphavirus 11 influenza 16 rickettsia
Bali	longitudinal fever study	outpatients (all ages)	92	24 flavivirus 6 alphavirus 30 influenza 6 rickettsiae
Denpasor,Bali	longitudinal fever study	inpatients (all ages)	133	36 flavivirus 13 alphavirus 18 influenza 6 rickettsiae
Sulawesi	longitudinal fever study	inpatients (pediatrics)	24	6 flavivirus
TOTAL			606	76 flavivirus 26 alphavirus 69 influenza 32 rickettsiae 1 mumps

Table 35

Arboviruses which might be encountered in Indonesia

Family	Genus	Antigenic Group	Virus	Hosts
Togaviride				
	Alphavirus	A		
			Bebaru (BEB)	man, mosquitoes
			Chikungunya*(CHIK)	man, birds, bats, mosquitoes
			Getah (GET)	man, pigs, mosquitoes
			Ross River*(RR)	man, birds, wallabies, mosquitoes
			Sagiyama*(SAG)	man, birds, domestic animals,mosquitoes
			Semliki Forest* (SF)	man, monkey, birds, mosquitoes
			Sindbis*(SIN)	man, birds, mosquitoes
			Whataroa (WHA)	man, birds, mosquitoes
	Flavivirus	B		
			Alfuy (ALF)	avians, mosquitoes
			Batu Cave (BC)	bats
			dengue*(DEN)	man, monkey, mosquitoes
			Edge Hill (EH)	wallabies, mosquitoes
			Japanese enceph-alitis*(JE)	man, domestic animals, bats, avians, mosquitoes
			Kokobera (KOK)	man, wallabies, kangaroos, mosquitoes
			Kunjin*(KUN)	man, birds, domestic animals, mosquitoes
			Langat (LGT)	rats, ticks
			Murray Valley enceph-alitis*(MVE)	man, domestic animals, mosquitoes
			Ntaya (NTA)	man, mosquitoes
			Sepik*(SEP)	domestic animals, mosquitoes
			Stratford (STR)	domestic animals, mosquitoes
			Tembusu (TMU)	man, mosquitoes
			Wesselsbron*(WSL)	domestic animals, mosquitoes
			West Nile*(WN)	man, domestic animals, birds, bats, monkeys, rats, mosquitoes, ticks
			Zika*(ZIKA)	man, mosquitoes

*causes disease in humans.

Family	Genus	Antigenic Group	Virus	Hosts
Bunyaviridae				
	Bunyavirus	Bunyamwera	Bunyamwera*(BUN)	man, monkey, rodents, mosquitoes
			Batai (BAT)	man, birds, rodents, domestic animals, moquitoes
			Wyeomyia*(WYO)	man, birds, mosquitoes
		California	Tahyna*(TAH)	man, birds, mosquitoes
		Koongol	Koongol (KOO)	domestic animals, mosquitoes
			Wongal (WON)	birds, bandicoot, mosquitoes
		Simbu	Aino (AINO)	mosquitoes
			Akabane (AKA)	man, mosquitoes
			Ingwavuma (ING)	man, domestic animals, mosquitoes
			Samford (SAM)	domestic animals, Culicoides
		Unassigned		
			Kaeng Khoi (KK)	man, bats, rats
		Bakau	Bakau (BAK)	man, monkeys, mosquitoes, ticks
		Mapputta	Mapputta (MAP)	mosquitoes
			Maprik (MPK)	mosquitoes
			Trubanaman (TRU)	man, domestic animals, Kangaroo, mosquitoes
		Turlock	Umbre (UMB)	man, birds, mosquitoes
		Nairobi sheep disease	Ganjam*(GAN)	man, ticks, mosquitoes
		Kaisodi	Lanjan (LJN)	rats, ticks

*causes disease in humans.

Family	Genus	Antigenic Group	Virus	Hosts
Rhabdoviridae				
	Undefined		Bovine ephemeral fever (BEF)	
Undefined				
		Quaranfil	Johnston Atoll (JA)	ticks
		Dera Ghazi Khan	Kao Shuan (KS)	ticks
		Sakhalin	Taggert (TAG)	ticks
		Ungrouped	Kowanyama (KOW)	domestic animals, kangaroos, mosquitoes
			Almpiwar (ALM)	reptiles
			Charleville (CHV)	phlebotomines
			Wongorr (WGR)	domestic animals, wallaby, mosquitoes
			Ngaingan (NGA)	domestic animals, wallaby, kangaroo, Culicoides

Table 36

Virus Working Stocks

Genus	Virus	Log_{10}TCID50/0.025 ml in Vero Cells
Alphavirus		
	Bebaru (BEB)	5.75
	Chikungunya (CHK)	7.0
	Getah (GET)	5.50
	Ross River (RR)	4.25
	Sagiyama (SAG)	4.75
	Semliki Forest (SF)	6.25
	Sindbis (SIN)	7.25
	Whataroa (WHA)	4.50
Flavivirus		
	dengue 2 (DEN-2)	4.0
	dengue 4 (DEN-4)	5.75
	Japanese encephalitis (JE)	5.75
	Kunjin (KUN)	3.0
	Langat (LGT)	1.50
	Tembusu (TMU)	4.25
Bunyavirus		
	Bunyamwera (BUN)	5.25
	Batai (BAT)	4.50

likely be the most frequently encountered etiologic agent. HI tests for flaviviral infections have already been completed and on those paired sera for which a diagnostic rise or presumptive standing antibody titer was measured, we will test in micro NT with the 4 serotypes of dengue. Those sera with flavivirus antibody, but which do not show a diagnostic increase in dengue NT antibody will be tested with other flaviviruses listed in Table 35.

Patients have also been tested for evidence of alphavirus infection by HI using CHIK, RR, and GET hemagglutination antigens (HA). Those paired sera will be tested for neutralizing antibodies using alphaviruses listed in Table 35. No tests for arthropod-borne viruses other than alphaviruses and flaviviruses have been completed.

The measurement of serum antibody in paired sera to other groups of viruses will be accomplished by indirect immunofluorescence. Patient sera showing a diagnostic rise in antibody titer from acute to convalescent phase will be tested in a micro NT test using representative viruses and antisera of the group selected from Table 36.

V. BIOCHEMICAL STUDIES

The unexpected discovery that Colorado tick fever (CTF) virus RNA has 12 segments was made early in 1980. The Orbivirus genus was first recognized as a distinct taxonomic group of arboviruses based upon the morphologic, serologic, and physicochemical data of viruses in the Yale Arbovirus Research Unit-WHO virus collection. Twelve distinct serogroups are recognized on the basis of CF cross-reactivity with one ungrouped set of viruses, and these are listed in Table 37. The Table lists 86 serotypes distinct by N test.

Double-stranded (ds) RNA segment analyses of CTF (D.L. Knudson). Results of ds RNA segment analyses by polyacrylamide gel electrophoresis indicated that representatives of serogroups of the orbiviruses had 10-segmented genomes (see 1978 and 1979 Annual Reports). Thus finding 12 segments in the CTF genome came as a surprise. The observation stimulated a more comprehensive study of the 4 other genera of Reoviridae as well as an in-depth study of different CTF isolates including clones of the Florio strain.

Representatives of the genera Rotavirus, cytoplasmic polyhedrosis virus group, and plant reovirus group were supplied as purified virus preparations; and the virus strains respectively included a human rotavirus isolated from a fecal sample, Bombyx mori cytoplasmic polyhedrosis virus, and wound tumor virus. Reovirus type 3 Dearing strain (Reovirus) was grown in L929 cells and purified following standard protocols. Changuinola, chosen to represent 10-segmented orbivirus RNA, and Colorado tick fever were grown in BHK-21 clone 13 cells and purified following the reovirus protocols. The dsRNA was purified from the virus samples following a SDS, chloroform-isoamyl alcohol extraction procedure. The dsRNA segments were end-labeled and separated by electrophoresis through a 10% acrylamide gel. Examination of an autoradiograph of the gel revealed characteristic separations of the dsRNA segments for each virus

Table 37
Orbiviruses

VIRUS	STRAIN
African horsesickness serogroup	
AHS 1	
AHS 2	
AHS 3	
AHS 4	
AHS 5	
AHS 6	
AHS 7	
AHS 8	
AHS 9	
Bluetongue serogroup	
BLU 1	
BLU 2	
BLU 3	
BLU 4	
BLU 5	
BLU 6	
BLU 7	
BLU 8	
BLU 9	
BLU 10	
BLU 11	
BLU 12	
BLU 13	
BLU 14	
BLU 15	
BLU 16	
BLU 17	
BLU 18	
BLU 19	
BLU 20	CSIRO 19
Changuinola serogroup	
Changuinola	BT 436
	BT 104
	BT 766
	BT 2164
	BT 2365
	VP 19A
	VP 46F
	VP 91E
	VP '41E
	CoA. 2837
Irituia	BeAn 28873
Gurupi	BeAr 35646
Ourem	BeAr 41067
Caninde	BeAr 54342
Altamira	BeAr 264277
Jamanxi	BeAr 243090

VIRUS	STRAIN
Colorado tick fever serogroup	
CTF	Condon
	Florio
	Florio MA
	65-68
	18
	D. occidentalis
Eyach	38
Corriparta serogroup	
Acado	EthAr 1846-64
Bambari	
Corriparta	MRM1
---	BeAr 263191
---	BeAr 295042
Epizootic hemorrhagic disease of deer serogroup	
EHD	Alberta
EHD	New Jersey
---	V1A
	V1B
	V2
	V3
---	IbAr 22619
---	IbAr 33853
Ibaraki	
---	IbAr 49630
---	XBM/67
Equine encephalosis serogroup	
7 serotypes	
Eubenangee serogroup	
Eubenangee	IN 1074
Pata	DakAr B 1327
Tilligerry	NB 7080
Kemerovo serogroup	
Baku	USSR LEIV 46A
Bauline	CanAr 14
	CanAr 63
	CanAr 128
	CanAr 133
	CanAr 172
	CanAr 174
Cape Wrath	ScotAr 20
Chenuda	EgAr 1170
	EgAr 1152

Table 37 continued
Orbiviruses

VIRUS	STRAIN
Fin isolates	Fin NorV-808
	Fin NorV-873
	Fin NorV-962
Great Island	CanAr 41
	CanAr 32
	CanAr 40
	CanAr 42
	CanAr 45
	CanAr 49
	CanAr 176
Huacho	CalAr 883
Kemerovo	R10
	R6
	R9
	R54
	KM3
	KM10
	L-75
	EgAn 1169-61
Kenai	71-1629
Lipovnik	Lip 91
	Lip 10
	Lip 11
	Lip 53
	Kol 42
	Kol 152
	Kol 154
	Kol 156
Mono Lake	CalAr 861
Mykines	DenAr 12
	DenAr 8
	DenAr 7
	DenAr 10
Nugget	AusMI-14847
O'khotskiy	USSR LEIV 287ka
Poovoot	
Seletar	SM-214
Sixgun City	RML 52451
Tindholmur	DenAr 2
	DenAr 3
Tribec	original
Wad Medani	EgAr 492
Yaquina Head	RML 56297-15
	RML 56297-45
	RML 56298-58
	RML 56298-59
	RML 56298-62
	RML 56298-90
---	BrestAr T222

VIRUS	STRAIN
Palyam serogroup	
Abadina	IbAr 22388
D'Aguilar	AusB 8112
Kasba	IG 15534
Nyabira	
Palyam	IG 5287
Vellore	IG 68886
Wallal serogroup	
Mudjinbarry	
Wallal	Ch 12048
Warrego serogroup	
Mitchell River	MRM 10434
Warrego	Ch 9935
Ungrouped	
---	AusGG 668
---	EthAr 3554
---	IbAn 57245
	IbAn 57204
	IbAn 57928
Japanaut	Mk 6357
Lebombo	
	SAAr 136
	SAAr 3896
	IbAn 22853
	IbH 26035
	IbAr 26808
Orungo	UgMp 359
	IbH 13019
	IbH 60818
	IbAr 11306
Umatilla	USA 69-V2161
---	USA T-50616

(Figure 1). The dsRNA profiles seen with reovirus, CPV, and WTV were analogous to those reported elsewhere. The WTV isolate exhibited a thirteenth, nonmolar, low molecular weight segment which suggests that the virus was not a wild type isolate. Variants of WTV have been observed, and this isolate may be a variant.

The dsRNA profile for Changuinola virus has ten well separated bands. In contrast, CTF virus has twelve equimolar segments of dsRNA (Figure 1). The molecular weights of the dsRNA segments were calculated using the reported values of reovirus 3D, and the data are presented in Table 38. CTF virus exhibits an apparent total molecular weight of 18 x 10^6 daltons which is significantly larger than those of the other genera.

Three additional isolates of CTF were similarly analyzed, and they also exhibited twelve bands. The isolate used in this study was CTF-Florio strain. The dsRNA profile of 24 clones of this isolate which were randomnly picked from plaques also yielded twelve segments, and one clone was plaque-purified three times in BHK-21 cells. A tissue culture virus stock was prepared of the multiply cloned virus, and a suckling mouse brain virus stock was also prepared. Identical dsRNA profiles were observed using both virus stocks as inoculum. Complement-fixation tests were also conducted using this cloned virus and NIH reference reagents. The results of these tests confirm that CTF-Florio strain virus belongs to CTF serogroup, Table 39.

This cloned stock of CTF virus is not a mixture of viruses with distinctive genotypes as assessed by its dsRNA profile, and its genome is composed of twelve segments of dsRNA which are present in equimolar concentrations. The genomes of three other isolates of CTF virus also exhibit twelve equimolar dsRNA segments. Thus, representatives of eleven of the twelve Orbivirus serogroups have ten segments of dsRNA, while the CTF serogroup is the exception with twelve segments. Since CTF virus is a novel member of the genus Orbivirus, additional characterization of the viral genome is needed. Then, a re-evaluation of its taxonomic status may be appropriate.

DsRNA analyses of Changuinola (CGL) serogroup viruses (O.F.P. de Oliva and D.L. Knudson). The Changuinola (CGL) serogroup viruses represent orbiviruses isolated in Central and South America and associated with phlebotomines in nature. CGL was first isolated from Lutzomyia spp. of sandflies and from human blood during a survey in the Almirante region of Bocas del Toro, Panama for arboviruses which lasted from 1959 to 1962. The human case was an individual who was employed as a mosquito catcher in the survey, and the case presented a mild febrile illness. CGL was serologically typed as a new agent, and it was partially characterized for its pathogenicity for mice and cell cultures. CGL was later demonstrated to be resistant to lipid solvents and to cross-react by complement-fixation with orbiviruses isolated from Oryzomys spp. and phlebotomines from Brazil. CGL was repeatedly isolated from the phlebotomines with habitats of swampforest and upland tropical rainforest ecologies during the survey. CGL isolates were made from phlebotomines in a subsequent survey for arboviruses in the Aguacate and Limbo regions of Panama over a

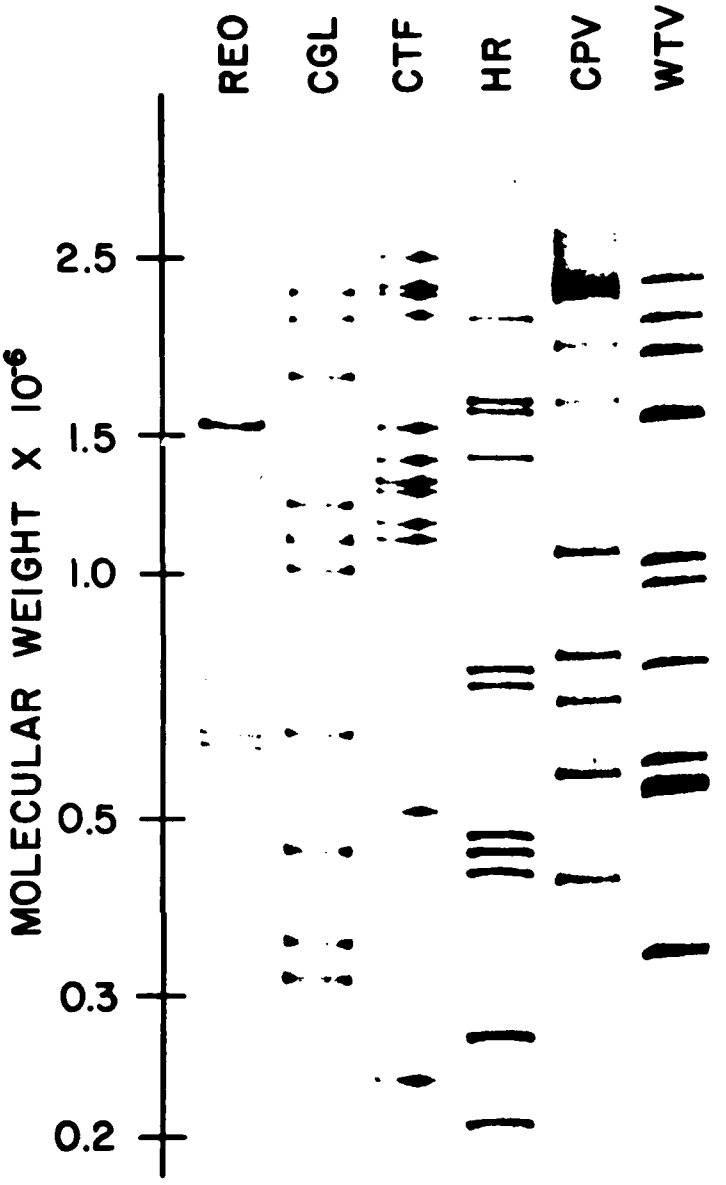

Figure 1. Autoradiogram of the genomes of representatives from the genera of Reoviridae depicting the segments for each virus and a relative molecular weight scale. The genera represented include Reovirus (Reovirus type 3 Dearing strain - REO), Orbivirus (Changuinola strain BT 436-CGL and Colorado tick fever Florio strain - CTF), Rotavirus (human rotavirus - HR), cytoplasmic polyhedrosis group (Bombyx mori cytoplasmic polyhedrosis -CPV), and plant reovirus group (wound tumor virus - WTV). Purified dsRNA was end-labeled at the 3'-end of the two strands of each segment by the addition of (5'-^{32}P)pCp (2000 to 3000 Ci/mmole, Amersham) using T4 RNA ligase. The samples were prepared for electrophoresis and run for 20 h at 20 mamps through 10% acrylamide. The gel was fixed, dried, and exposed to Kodak NS-5T X-ray film. The molecular weight scale was calculated using the reported values of reovirus.

Table 38. Observed Molecular Weight of dsRNA Segments of Members of Reoviridae

Segment	Molecular weight x 10^{-6} daltons*					
	REO	CGL	CTF	HR	CPV	WTV
1	2.59	2.29	2.53	2.14	2.36	2.37
2	2.42	2.12	2.36	1.70	2.32	2.13
3	2.29	1.79	2.29	1.64	2.27	1.94
4	1.57	1.24	2.17	1.44	1.98	1.62
5	1.57	1.12	1.57	0.79	1.69	1.62
6	1.36	1.04	1.43	0.75	1.09	1.06
7	0.93	0.65	1.33	0.49	0.81	0.99
8	0.75	0.46	1.30	0.46	0.71	0.79
9	0.65	0.36	1.19	0.44	0.58	0.60
10	0.62	0.32	1.13	0.28	0.43	0.56
11			0.52	0.22		0.55
12			0.24			0.34
total	**14.75**	**11.39**	**18.06**	**10.35**	**14.24**	**14.57**

*The molecular weights of the segments were calculated by regression analysis using the reported values of reovirus 3D. The virus abbreviations are explained in the legend of figure 1.

Table 39. Complement Fixation Tests of Plaque-purified Colorado Tick Fever Virus

Antigens	**Mouse Ascitic Fluid**		
	CTF	NIH-CTF	Normal
CTF (Condon strain)	128/>512	128/>512	<4/<4
CTF (Florio plaque-purified strain)	64/128	128/256	<4/<4

Complement-fixation tests were done using sucrose-acetone extracted suckling mouse brain antigens. CTF (Condon strain), CTF immune mouse ascitic fluid, and normal mouse ascitic fluid were from the Yale Arbovirus Research Unit, WHO Reference Center for Arboviruses. The NIH-CTF mouse ascitic fluid was supplied by National Institutes of Health Reference Reagents (Cat. No. V506-701-562). The data are expressed as reciprocal titers of antiserum/antigen.

period of 1969 to 1971, and again, the virus was repeatedly isolated. CGL virus has been isolated in Panama from phlebotomines, mosquitoes, and Bradypus sloths over the past eighteen years (P.H.Peralta, 1979, personal communication). These isolates are listed in Table 40. Complement-fixation tests were used to characterize some of these isolates as CGL (Table 41). CGL (BT-436), BT-104, and BT-2164 were all collected from the Almirante site, and yet, they represent distinct serotypes. Thus, the actual number of distinct serotypes remains to be demonstrated. However, there are at least eight.

This study was undertaken to examine the extent of genetic variation of the members of the CGL serogroup as assessed by PAGE analyses of the 10 segments of dsRNA. Furthermore, two new Corriparta group isolates from Brazil sent to YARU by Dr. F. Pinheiro (Belem, Brazil) in 1978 were also examined. Table 42 lists the viruses which were analyzed, and it also provides information on the history of the isolates.

The CGL isolates chosen represent viruses which were isolated at independent sites. Multiple isolations were made at the two sites, but there was a period of eight to ten years between isolations. The isolates from the Almirante site are also representative of those which have been analyzed by neutralization tests (Tables 43 and 44). CGL isolates BT-436 and BT-766 as well as BT-2164 and BT-2365 were indistinguishable by neutralization tests. The isolates from Aguacate react in CF tests with CGL, but neutralization tests have not been performed. The remaining CGL serogroup viruses are distinct in neutralization tests, and hence, they represent serotypes of the CGL serogroup. Three members of the Palyam (PAL) serogroup were also included in these analyses (Table 42).

PAGE analyses of the dsRNA of these CGL viruses yielded dsRNA profiles which correlated with the neutralization data (Figure 2). For example, the profiles for BT-436 and BT-766 were indistinguishable. Likewise, BT-2164 and BT-2365 yielded similar profiles. Although BT-104 was similar to BT-436 and BT-766, minor differences in segment mobilities were seen. Thus, the Almirante site yielded three distinct genotypes. In contrast, the Aguacate site isolates resulted in four distinct genotypes, and these genotypes were distinct from the three found with the Almirante isolates. The remaining four CGL isolates from Colombia and Brazil exhibited four new genotypes. Thus, eleven distinct profiles or genotypes were observed.

PAGE analyses of the two new Brazilian Corriparta group isolates revealed that each had a distinct profile. Likewise, the three PAL serotypes exhibited distinctive genotypes (Figure 3). A comparison of the PAL genotypes with the Brazilian genotypes suggested that the profiles between those two groups were not even generally similar.

Cloning of Tribec (TRI) virus (R. Lavendar and D.L. Knudson). Since the dsRNA profile of TRI exhibited twelve segments, the TRI virus stock was either a mixture of viruses or the genome had twelve dsRNA segments which were nonmolar in concentration. Thus, TRI should be a mixture of four distinct genotypes as assessed by dsRNA profile analyses. The TRI virus stock was cloned by a plaque-picking procedure using BHK-21 cells. The

Table 40. Changuinola serogroup isolates from Panama at GMI

Locale	Province	Year	Isolates Source	Number
Almirante	Bocas del Torro	1959-61	Lutzomyia	120
		1959-61	Human	1
Aguacate	Panama	1969-71	Lutzomyia	120
		1974	Bradypus	1
Limbo	Canal Zone	1969-71	Lutzomyia	19
Cerro Nique*	Darien	1972	Lutzomyia	several
Maje	Panama	1973-75	?	?
		1976	Anopheles	1
		1976	Lutzomyia	4
		1976	Mansonia dyari	1
		1977	M. dyari	2
Cerro Azul	Panama	1975	Bradypus	2
Chilibre	Panama	1976	Bradypus	1

*Suspected orbiviruses because the CFT showed a low level crossreaction.

Table 41. Changuinola Serogroup Viruses: Complement-fixation Tests

Antigen		Serum or Ascitic Fluid			
		BT 436	BeAn28873	BeAr35646	BeAr41067
Changuinola	BT 436	64	32	8	32
Irituia	BeAn 28873	16	128	16	32
	BeAr 35646	16	128	16	32
	BeAr 41067	32	32	8	32

Reciprocal of dilution giving equal to or greater than 50% fixation.
Data are from Borden et al. (1971). J. gen. Virol. **13**, 261-271.

Table 42. Changuinola and Palyam Serogroup Viruses

Isolate Designation		Isolate History					
		Country	Site	Year	Source	Passage	
Changuinola serogroup:							
	BT 104	Panama	Almirante	59-61	Lutzomyia spp.	SMB_{38}	BHK_1
Changuinola	BT 436	Panama	Almirante	3-7.i.60	Lutzomyia spp.	SMB_3	BHK_1
	BT 766	Panama	Almirante	59-61	Lutzomyia spp.	SMB_{40}	BHK_1
	BT 2164	Panama	Almirante	59-61	Lutzomyia spp.	SMB_{15}	BHK_1
	BT 2365	Panama	Almirante	59-61	Lutzomyia spp.	SMB_{17}	BHK_1
	VP 19A	Panama	Aguacate	15.v.69	Lutzomyia spp.	$VERO_3$	SMB_1
	VP 46F	Panama	Aguacate	19.vi.69	Lutzomyia spp.	$VERO_3$	SMB_1
	VP 91E	Panama	Aguacate	27.vii.69	Lutzomyia spp.	$VERO_2$	SMB_1
	VP 141E	Panama	Aguacate	12.xii.69	Lutzomyia trapidoi	$VERO_4$	SMB_1
Irituia	BeAn 28873	Brazil	km 92*	29.iii.61	Oryzomys spp.	SMB_9	
	BeAr 35646	Brazil	km 94*	ix.61	Lutzomyia spp.	SMB_4	BHK_1
	BeAr 41067	Brazil	km 87*	iv.62	Lutzomyia spp.	SMB_4	BHK_1
	CoAr 2836	Colombia	Rio Raposo	vi-vii.64	Lutzomyia spp.	SMB_7	BHK_1
Palyam serogroup:							
Abadina	IbAr 22388	Nigeria	Ibadan	2.ix.67	Culicoides spp.	SMB_{11}	
D'Aguilar	Aus B 8112	Australia	Bunya, Qld.	2.iv.68	Culicoides brevitarsis	SMB_3	BHK_1
Palyam	IG 5287	India	Vellore	28.vi.56	Culex "vishnui"	SMB_2	BHK_1
Corriparta serogroup							
	BeAr 295042	Brazil	Jacareacanga	30.i.76	Culex melanocion spp.	SMB_8	
	BeAr 263191	Brazil	Belem	11.x.79	Culex declarator	SMB_6	

*Kilometer marker of the Belem-Brasilia Highway.

Table 43. Changuinola Serogroup Viruses: Neutralization Tests Performed at the Middle America Research Unit

Virus		Antiserum						
		BT104	BT436	BT2380	VP202A	BeAn28873	BeAr41067	BeAr54342
Changuinola	BT 104	64	0	0	0	0	0	0
	BT 436	0	256	0	0	0	0	0
	BT 2380	0	0	16	0	0	0	0
	VP 202A	0	0	0	256	0	0	0
Irituia	BeAn 28873	0	0	0	0	1024	0	0
	BeAr 41067	0	0	0	0	0	256	0
	BeAr 54342	0	0	0	0	0	0	256

Reciprocal of highest serum dilution giving 90% plaque reduction in Vero cultures. Four-fold serum dilutions were used. Data are courtesy of Dr. R. Tesh.

Table 44.

Changuinola Serogroup Viruses: Neutralization Tests Performed at the Yale Arbovirus Research Unit

Virus		Serum								
		BT104	BT436	BT766	BT2164	BT2365	BeAn28873	BeAr35646	BeAr41062	CoAr2837
Changuinola	BT 104	32	0	0	0	2	0	0	0	0
	BT 436	0	256	256	0	0	0	0	0	0
	BT 766	4	256	256	2	4	0	0	0	0
	BT 2164	0	0	0	32	32	0	0	0	0
	BT 2365	0	0	0	128	64	0	0	0	0
Irituia	BeAn 28873	0	0	0	0	0	256	0	0	0
	BeAr 35646	0	0	0	0	0	0	256	8	0
	BeAr 41067	0	0	0	0	0	0	0	256	0
	CoAr 2837	0	0	0	0	0	0	2	0	256

Reciprocal of serum dilution giving 50% or greater plaque inhibition in Vero cultures (S.T. Baker, M.D. and M.P.H. Thesis, Yale University School of Medicine, 1967).

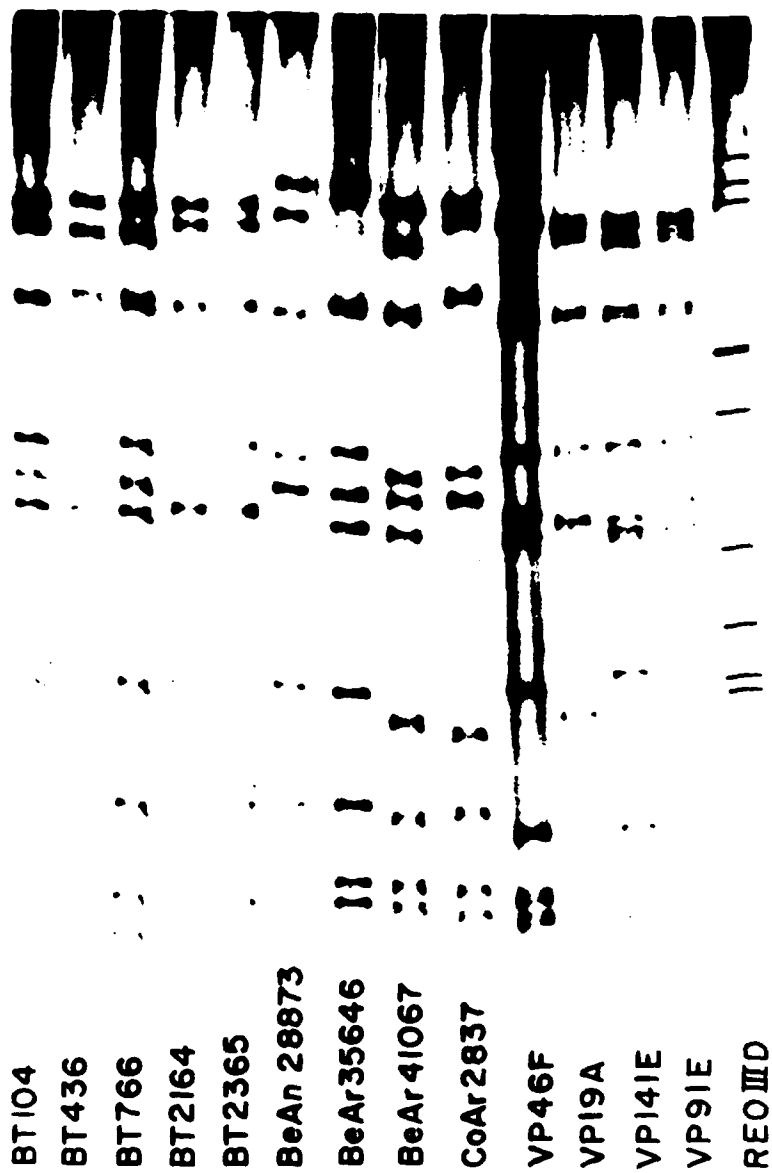

Figure 2. PAGE analysis of the dsRNA segments of each of the virus isolates tested. The segments are in order of decreasing molecular weights. The abbreviations for the gel tracks are found in table 42.

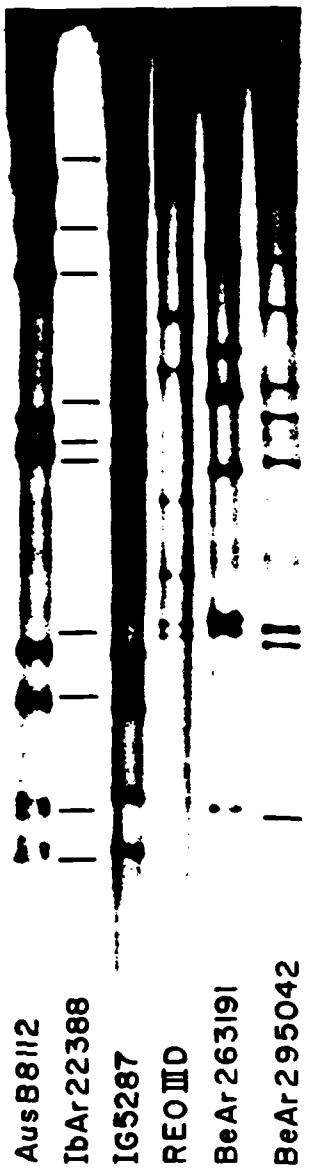

Figure 3. PAGE analysis of the dsRNA segments of each of the virus isolates tested. The segments are in order of decreasing molecular weights. The abbreviations for the gel tracks are found in table 42.

dsRNA profiles were examined from over a hundred clones in attempt to isolate the four genotypes and to determine their frequency in the original virus stock. Figure 4 represents an autoradiogram of the results for twenty clones. The four predicted genotypes were isolated, but their frequency of appearance differed for the genotypes. Three of the four genotypes were observed with equal frequencies, and one was less frequent. Thus, the TRI virus stock was a mixture of at least four genotypes each with a distinct dsRNA profile.

DsRNA analyses of other orbiviruses (D.L. Knudson). DsRNA profiles have been examined for most of the orbiviruses and many of their respective strains as listed in Table 37. The twenty serotypes of BLU virus have been recently analyzed in collaboration with the U.S.D.A. Plum Island Animal Disease Laboratory (PIADL) at Plum Island, NY. Again, distinct dsRNA patterns were observed (data not shown).

The Kemerovo-Cape Wrath complex strains which include; Bauline, Cape Wrath, Fin isolates, Great Island, Mykines, Okhotskiy, Tindholmur, and Yaquina Head have been analyzed for their dsRNA patterns. Each serotype was distinct, and strains of a given serotype exhibited generally similar profiles with minor variations in segment mobilities. For example, the seven virus strains isolated from Great Island, a restricted ecosystem, show differences in their profiles such that four distinct genotypes were observed (data not shown).

Several of the ungrouped viruses have been analyzed, and their dsRNA patterns demonstrated that they were orbiviruses because they had ten segments of dsRNA. For example, Orungo virus from Africa exhibited ten dsRNA segments (data not shown). Orungo virus is known to be associated with febrile illness in man, and serosurveys of the Nigerian population have demonstrated CF antibody in more than 60% of those tested.

The overwhelming feature that these studies reveal is that there is extensive genetic variation in orbiviruses as assessed by their dsRNA patterns of the viral genomes. These data suggest that the majority of the isolates exhibit distinct RNA profiles of ten segments which is consistent with their classification as orbiviruses. There are instances where segments appear to co-run, and as a result, some isolates seem to have less than 10 segments. When the autoradiograms were analyzed by scanning densitometry, the segments which co-ran were noted. The RNA profiles of strains isolated from a specific geographic region were often indistinguishable. In a few instances, variation in a single segment between two strains was also seen. In general, viruses which originated from specific areas were similar, but extensive variability in the dsRNA profiles was observed for isolates from different regions. All the Orbivirus serogroups, except CTF, have genomes which are composed of ten segments.

VI. IMMUNOLOGY AND PATHOGENESIS STUDIES

Effect of immunosuppression on the pathogenicity of the 2-8 attenuated strain of Japanese encephalitis virus (B.Q.Chen and

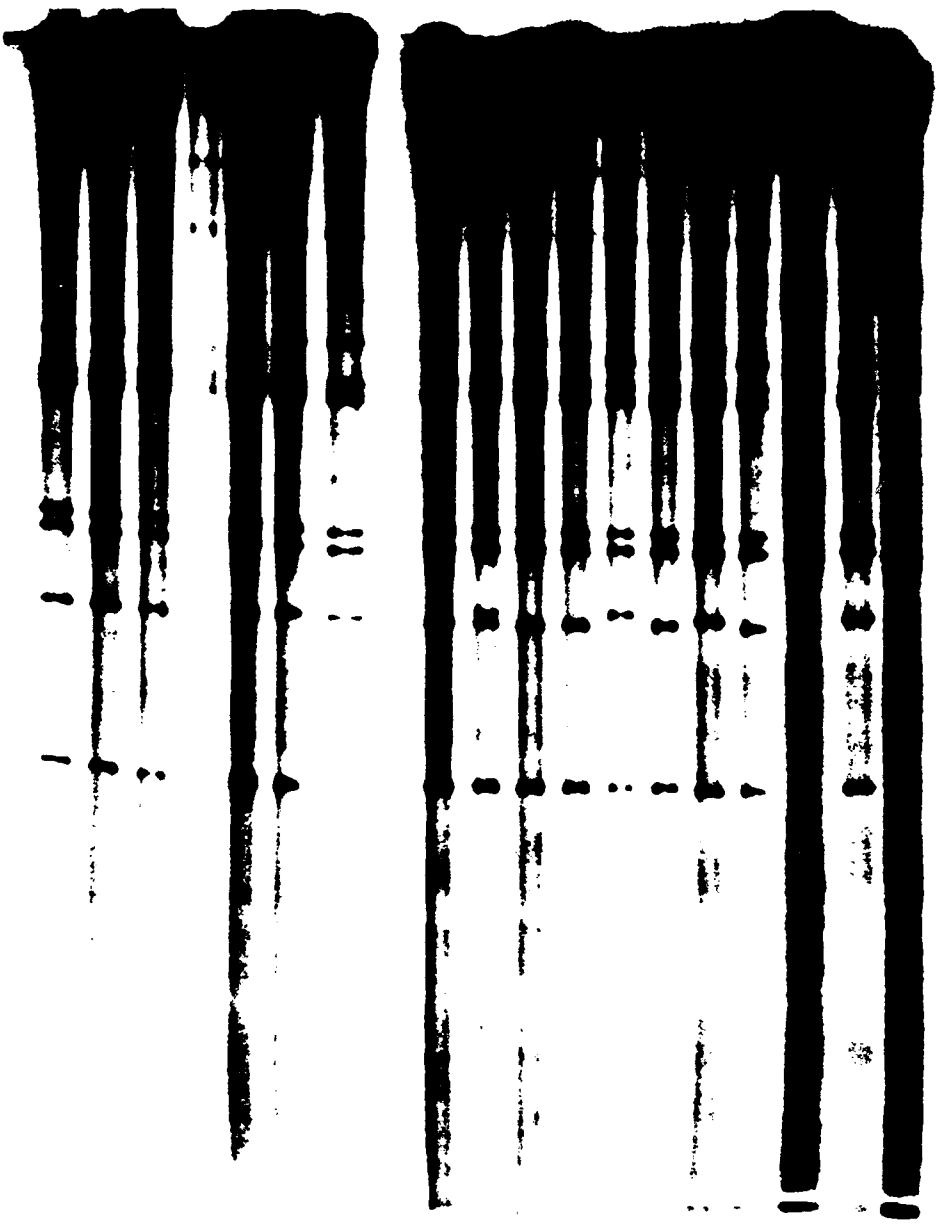

Figure 4. DsRNA profiles of Tribec clones. Four distinct genotypes were observed when Tribec clones were analyzed by PAGE. The genomic variants found show different mobilities for segments 3 and 9 (numbered from the top of the figure). An example of a mixture of genotypes as a result of inadequate cloning is shown by the gel track in the middle where two segments are seen at position 9.

G.H.Tignor). In an attempt to understand how the 2-8 strain of Japanese encephalitis (JE) virus is attenuated for mice, the 2-8 strain and the virulent parent SA14 strain were studied in outbred Swiss mice, and in CBA/J, and C3H/RV and C3H/HE inbred mice before and after cyclophosphamide immunosuppression. Mice were immunosuppressed by ip administration of 150 mg/kg cyclophosphamide on days 1, 3, and 5 after virus inoculation.

Two mice were sacrificed at 1 hr, 3 hr, 5 hr, 8 hr, 24 hr, 3 days, 5 days, and 8 days after inoculation of virus. The following were collected: blood, brain, spleen, and bone marrow. In some experiments kidney and liver were also sampled. The viruses were assayed in BHK-21 cells. Tissues were also examined by immunofluorescence.

JE 2-8 attenuated virus. The titer of 2-8 JE virus inoculum was 9.3 $\log_{10}$ $TCID_{50}$/ml. C3H/HE mice inoculated ic or ip did not die or become ill. Mice which survived ic inoculation had HI antibody 14 days later; these mice survived challenge on the 14th day with fully virulent SA 14 virus (8.5 $\log_{10}$ $TCID_{50}$ml). After ip inoculation, virus isolated from blood was of higher titer (6.5 $\log_{10}TCIF_{50}$/ml) than virus isolated from other organs until 8 hrs after injection. Virus was not isolated from the brain or bone marrow after 8 hrs, but was isolated from the spleen and blood at 3 days, but not at 5 days or 8 days.

C3H/RV (flavivirus resistant) mice inoculated ic or ip with 2-8 virus did not die and did not become ill. HI antibody was not detected 14 days later in mice inoculated ip, but these mice survived ic challenge with fully virulent SA14 virus (8.5 $\log_{10}$ $TCID_{50}$ml). After ip inoculation, virus isolated from the blood was of higher titer (6.5 $\log_{10}TCID_{50}$/ml) than virus isolated from other organs until 8 hrs after injection. The viremia ended at 8 hr. Virus was not isolated from the brain at or after 3 hr in contrast to the results in C3H/HE mice. Virus was isolated from bone marrow and spleen at 24 hr, but not at 3 days, in contrast to the results in C3H/HE mice.

Two of 10 cyclophosphamide-treated C3H/RV mice infected ic with 2-8 virus died whereas 0/10 untreated mice died. The control group given drug alone survived.

The mortality in CBA/J mice inoculated ic was 2/10; mice inoculated ip survived. The results of cyclophosphamide treatment were quite different from the results in C3H/RV mice in that immunosuppression caused death of 10/10 mice inoculated ic and 10/10 mice inoculated ip. There were no deaths among control mice given cyclophosphamide alone. Mice injected ip with 2-8 virus (8.5 $\log_{10}TCID_{50}$/ml) and treated with cyclophosphamide were reconstituted with bone marrow, spleen cells, thymus cells, or blood taken from donor mice which had been immunized 1 month before. Recipients of bone marrow cells survived and 8/10 recipients of spleen cells survived. Four of 10 mice given thymus cells survived while all mice died given 1.0 ml of serum which had an HI titer of 1:64.

Random bred Swiss mice infected with 2-8 virus by either the ic or ip route of inoculation did not die. After ip inoculation, virus was isolated

from the blood up to 5 hr after injection. Virus was absent from the brain after 3 hr, but was isolated from the spleen, kidney, bone marrow and thymus up to day 3. Virus persisted in the bone marrow through day 8 when sampling ended. The effect of immunosuppression of random bred mice after ip injection of virus was the same as CBA/J mice in that 10/10 infected and cyclophosphamide-treated mice died. There was no mortality observed among control mice given drug alone, or among infected and untreated mice. Immunofluorescence antigen in the brains of immunosuppressed mice was widely distributed. In contrast, only isolated foci of infected cells were found in the brains of infected and untreated mice.

JEBV SA-14 Virus. The dose of SA 14 virus was 9.0 $\log_{10}$TCID$_{50}$/ml. Six of 6 C3H/HE (flavivirus susceptible) mice inoculated ic died, while 10/10 mice inoculated ip died. The virus titer in the blood of mice inoculated ip was at higher (6.5 $\log_{10}$TCID/$_{50}$) than in other organs until 8 hrs. At 24 hrs and later, virus was isolated from the spleen and brain, but not from the bone marrow or blood.

The mortality among C3H/RV (flavivirus resistant) mice inoculated ic was 7/16; 0/10 mice inoculated ip died. Those mice which survived ip inoculation of virus also resisted challenge ic 14 days later with homologous SA 14 virus (8.5 $\log_{10}$TCID$_{50}$/ml). Viremia of mice inoculated ip was of a higher titer (6.5 $\log_{10}$TCID/$_{50}$) than virus in other organs until 8 hrs. Virus was not isolated from the bone marrow at 24 hrs and 3 days; virus was isolated from the spleen at 24 hrs only.

The mortality among C3H/RV (resistant) mice inoculated ic and treated with cyclophosphamide was 10/10. Two of 10 infected and untreated mice died; controls given drug alone survived. After ip inoculation of virus, 0/10 infected and untreated mice died while 6/10 infected and treated mice died.

All CBA/J and random bred Swiss mice inoculated ic or ip with SA 14 virus died. In random bred Swiss mice there was a biphasic viremia in that virus was isolated from the blood at one hr, was not isolated at 3 hr, but was isolated from the blood at 5 hr until death. In general, virus isolated from the blood was at lower titer than virus isolated from the brain, spleen and bone marrow.

Discussion. Did the JEV 2-8 vaccine have an intrinsically lower affinity for neuronal cells than did the parent virus? The answer to the question depended to a large extent upon the mouse strain used in the comparative study. JEV 2-8 vaccine had a lower intrinsic affinity for neuronal cells in C3H mice. The conclusion is based on the following observations. First, C3H/HE (flavivirus susceptible) and C3H/RV (flavivirus resistant) mice were equally resistant to ic and ip infection with JBEV 2-8 vaccine. In contrast, JEV SA-14 virus killed C3H/HE mice by ip and ic inoculation. Secondly, immunosupression did not significantly modify the resistance of C3H/RV mice infected ic with 2-8 virus whereas it increased mortality in C3H/RV mice infected ic with SA-14 virus.

Although JBEV 2-8 vaccine did not kill immunocompetent CBA/J or

random bred Swiss mice by either the ic or ip route of inoculation, the resistance was clearly immunologic since immunosuppressed CBA/J mice inoculated ic or ip and immunosuppressed Swiss mice inoculated ip died with an encephalitic illness. Immune bone marrow cells and, to a lesser extent, spleen cells restored resistance to immunosuppressed mice infected ip thereby confirming that cells of the reticuloendothelial system are important in mediating protection. Interestingly, antibody from the same donor mice did not confer protection upon virus-infected recipients.

Pathogenesis of attenuated 2-8 strain of JE virus in Culex tritaeniorhynchus (B.Q. Chen and B.J. Beaty) The attenuated 2-8 strain of JE virus and the parent SA 14 strain which had been passaged 14 times ic in baby mice, were tested for their ability to infect and be transmitted by C. tritaeniorhynchus. The mosquitoes were obtained from R. Baker of the University of Maryland, maintained in colony at YARU, and used at 5-10 days after emergence. Both viruses were grown and assayed in BHK-21 cells.

Intrathoracic inoculation. Maximum titers of both 2-8 and SA 14 viruses were achieved in from 4 to 6 days after intrathoracic inoculation. The SA 14 virus grew to 10^8 $TCID_{50}$/mosquito in two experiments. The 2-8 attenuated strain replicated equally well in one experiment and about 2 log $TCID_{50}$ less in a second experiment. In this latter experiment mosquitoes transmitted the SA 14 strain to mice in 16/16 attempts, but failed to transmit the 2-8 strain as measured by death of baby mice (Table 45). Tests are pending of the sera of surviving mice to see if antibody was produced.

Mosquitoes fed on SA 14 and 2-8 viruses were tested on days 12, 14 and 15 after feeding, for virus content of the whole triturated mosquito. Small amounts of 2-8 virus were detected; between 3.5 and 8.5 log $TCID_{50}$/mosquito were found in SA 14-infected insects (Table 46). In transmission experiments using orally-infected mosquitoes, 15/20 (75%) of SA 14-infected mosquitoes transmitted; 0/21 of the 2-8 infected mosquitoes transmitted as judged by death of exposed baby mice (Table 47).

The results are interpreted to mean that there is a defect in the 2-8 virus at the level of the gut barrier. There also may be a defect at the salivary gland level, but additional experiments are needed to confirm this.

Pathogenesis of two strains of CTF for mice (C. Reed, D.L. Knudson, and G.H. Tignor). Two strains of CTF virus, a Florio mouse adapted isolate (CTF-FMA) and a wild isolate (CTF-18), were inoculated into weanling mice intracerebrally (ic). There were no obvious changes in the CTF-18 inoculated mice, but the condition of the CTF-FMA mice deteriorated and resulted in death by day 7 post inoculation (p.i.). The amount of virus present in the brains of CTF-FMA mice was 10^4 PFU/ml or greater on day 1 p.i. The titer of virus continued to rise to a peak on day 4 and 5. Some mice inoculated with CTF-18 did not have demonstrable virus in the brain at almost any time in the experiment. The virus titers were not consistent between mice on any day nor between days, and were generally lower than those for CTF-FMA inoculated mice.

Table 45

Viral transmission rate of mosquitoes infected by intrathoracic infection with JE vaccine 2-8 strain and its parent virulent SA 14 strain

Virus and Dose	Extrinsic incubation (days)	Number of mice bit sucessfully by mosquito	Number of mice dead	Viral transmission rate
JE SA 14 $TCID_{50}$ Log 9.0/ml	10	16	16	100%
JE 2-8 $TCID_{50}$ Log 9.0/ml	10	16	0	0%

Table 46

Viral titer of each mosquito fed artificially by JE vaccine 2-8 strain and its parent SA 14 strain after different days of incubation

Virus and dose	Extrinsic incubation (days)	Number of mosquito	Viral titer of each mosquito (Log $TCID_{50}$/ml)
JE SA 14 $TCID_{50}$ Log 6.67/ml	12	Nov-13-2	7.0
		-3	8.5
		-4	8.5
		-5	8.0
		-6	8.5
		-7	8.0
		-9	6.0
		-11	8.0
	14	Nov-II-I	8.5
		-2	4.5
	15	Nov-13-I	4.5
		-3	8.5
		-6	8.5
		-7	4.0
		-8	4.0
		-9	4.0
		-10	6.0
		-11	3.5
		-14	8.0
		-15	8.0
JE 2-8 $TCID_{50}$ Log 7.5/ml	12	Nov-13-I	2.5
		-2	2.5
		-3	2.0
		-4	2.0
		-8	0.5
	14	Nov-II-I	0.5
		-3	0.5
		-4	0.5
		-5	0.5
		-8	0.5
	15	Nov-13-I	0.5
		-2	0.5
		-3	0.5
		-4	0.5
		-5	0.5
		-6	0.5
		-7	0.5
		-8	0.5
		-9	0.5
		-10	0.5

Table 47

Transmission to mice by mosquitoes fed artificially with JE vaccine 2-8 strain and its parent virulent SA 14 strain after different days of incubation

Virus and Dose	Extrinsic incubation (days)	Number of mice bit successfully by mosquitoes	Number of mice dead	Viral transmission rate of mosquitoes fed with JE virus
JE SA 14 $TCID_{50}$ Log 6.67/ml	12	11	8	
	14	2	1	
	15	7	6	
		20	15	75%
JE 2-8 $TCID_{50}$	12	9	0	
	14	6	0	
	15	6	0	
		21	0	0

The principal site of antigen location and histopathologic lesions in the brain were different for the two strains. The major sites were in the telencephalon and diencephalon for CTF-FMA and in the cerebral cortex and pons for CTF-18. The distribution of antigen is consistent with a hypothesis that viral spread within the brain occurred in part via nerve pathways, and that the pathways were different for the two strains.

Studies of Phlebotomus fever group viruses and their reassortants for increased pathogenicity (R.E.Shope). Cloned wild-type viruses and reassortants in the Phlebotomus fever group are being supplied by the University of Alabama to test for enhanced pathogenicity in small laboratory animals. To date no increased pathogenicity, as manifested by change in ability to kill mice by peripheral inoculation or decreased average survival time, has been noted.

VII. DEVELOPMENT OF NEW TECHNIQUES

The enzyme-linked solid phase immunoassay for antibody (J. Meegan)
The enzyme-linked immunosorbent assay (ELISA) has been used for detection of antibody and antigen with a number of viruses representing several serological groups (see previous Annual Reports). The ELISA was extended to the detection of antibodies to Rift Valley fever (RVF) viruses in human sera (previously collected for diagnostic or survey purposes).

The RVF ELISA was developed using inactivated antigens. Virus bands recovered after ultracentrifugation (potassium tartrate-glycerol gradients) of either formalin inactivated tissue culture vaccine or infected mouse liver yielded excellent ELISA antigens. In convalescent human sera, antibody titers (read with a spectrophotometer) were comparable to neutralization titers and ranged from 1/10,000 to 1/50,000. Preliminary experiments using a double antibody method (antigen + unknown human serum + goat antihuman + enzyme conjugated anti-goat) improved specificity and sensitivity. Additionally, employing a sandwich method (mouse anti-RVF + antigen + unknown human sera + enzyme conjugated goat antihuman) appears to improve the ELISA to levels comparable to the double antibody method. Other experiments in progress involve the enzyme-linked fluorescent assay (ELFA) which appears to extend the sensitivity of the ELISA test and makes visual reading comparable to spectrophotometric analysis. Currently we are employing these tests to determine the time of onset and magnitude of antibodies in serial sera collected from patients with documented RVF. This collection of sera has been analysed by neutralization, hemagglutination-inhibition, complement-fixation and indirect-fluorescent antibody tests, and tested for IgG and IgM antibody response. To date, ELISA appears to parallel the neutralizing antibody response; further comparisons, and IgG and IgM antibody detection are in progress.

Parameters affecting the ELISA test also have been studied. Increasing the purity of viral antigens improves the sensitivity and specificity of the test, but the process of attaching the antigen to the solid phase (polystyrene plate) remains undefined and variable. We have developed an ELISA utilizing antigen covalently bound to a solid phase

(either agarose or polyacrylamide microspheres). Our preliminary results show these antigen-spheres can be used as antigen in the HI, ELISA, or IFA tests (and presumably will function in the CF and radioimmunoassay tests). Current studies are designed to perfect this multitest antigen and determine its ability to be lyophilized, reused, and/or employed in a polyantigen ELISA test.

ELISA to detect La Crosse antigen in mosquito pools (S. Hildreth, C. Frazier, and B.J.Beaty). The ELISA system was investigated as a possible tool to detect arbovirus antigens within mosquito pools of various sizes. The particular ELISA system used was the indirect sandwich method; comprised of a capture viral specific antibody attached to a plastic surface, followed by the test sample then by a detector viral specific antibody and then the anti-species enzyme conjugate.

Aedes triseriatus were infected intrathoracically with 20 pfu of La Crosse (LAC) virus, harvested at 15 days post-inoculation and stored at -70°C. Each mosquito was thawed and headsquashed then tested for LAC antigen by fluorescent antibody (FA) method, both direct and indirect. Mosquitoes proven FA (+) were used individually or combined with normal mosquitoes to produce various pool categories (see Table 48). The diluent for the mosquito pools was 0.02M PBS with 10% newborn calf serum and 1% penicillin-streptomycin mixture. Mosquito samples were ground with a mortar and pestle with 1.0 ml of diluent, samples were centrifuged at 800 x g for 20 min and the supernatant collected and stored at -70°C. Immediately before testing, tween -20 was added to mosquito pools to a final concentration of 0.05%.

Mouse anti-LAC antibodies were purified by Sephadex G-200 column chromatography, diluted 1:50 in 0.6 M carbonate buffer and 0.2 ml was coated to the microtiter wells (Microelisa substrate plates, Dynatech Labs.,Inc.) by overnight incubation at 4°C. Plates were washed 4 times with 0.02 M PBS-tween 20 (0.05%). Mosquito pool preparations were added to the wells (0.2 ml) and incubated for 2 hours at room temperature. Plates were again washed 4 times with PBS-tween and the detector antibody (rabbit anti-LAC $(NH_4)_2SO_4$ precipitated Ig fraction, 1:400) was added. Following an additional 2 hour incubation (room temperature) the plates were again washed and the conjugate was added (goat anti-rabbit IgG-alkaline phosphatase, 1:750). Plates were incubated 2 more hours at room temperature. After washing, the substrate (p-nitrophenyl phosphate), 1 mg/ml in diethanolamine buffer, was added. After approximately 30 min the reaction was stopped with 3 N NaOH. Plates were read using a Titertek Multiskan (Flow Laboratories) microtiter plate reader. Replicate samples were produced for most mosquito pools. The mean of the mosquito control, normal mosquitoes in a pool of approximately the same size, served as a reference for evaluating other samples. As such, a test sample was considered positive for LAC antigen if the absorbance value was greater than 3 standard deviations from the mean of the mosquito control, matched on pool size and dilution of sample.

The possibility that mosquito tissues or viral aggregates could reduce the sensitivity of the ELISA test was examined. Selected pools,

Table 48

Antigen detection in mosquito pools[a]

Sample description					Mean absorbance values dilutions			
Viral status of pool	No. of infected	No. non-infected	Total mosquitoes	No. of replicates	10^{o}	10^{-1}	10^{-2}	
LAC	1	0	1	3	.697	.916	.575	
LAC	2	0	2	3	.680	.900	.540	
Control	0	1	1	3	.414(.0396)	.411(.0438)	.435(.0558)	trial 1
Control	0	2	2	3	.378(.0183)	.385(.0129)	.424(.0125)	
Control	PBS-tween		0	6	.424(.0127)			
LAC	tissue culture fluid		0	1	1.340	1.188	0.772	
Modoc	tissue culture fluid		0	1	0.366	0.379	0.392	
LAC	1	10	11	2	.450	.426	243	
LAC	2	10	12	1	.375	.270	.196	
LAC	3	10	13	1	.393	.629	.467	
LAC	1	50	51	3	.698	.659	.295	
LAC	3	50	53	2	.691	.750	.448	
JC	5	5	10	1	.217	.303	.245	trial 2
JC	2	10	12	1	.165	.223	.208	
JC	1	0	1	1	.227	.258	.219	
Control	0	2	2	2	.201(.0107)	.217(.0269)	.219(.0346)	
Control	0	10	10	3	.154(.0308)	.163(.0300)	.198(.0250)	
Control	0	50	50	3	.199(.0457)	.228(.0390)	.186(.0220)	
Control	PBS-tween		0	3	.181(.0027)			
LAC	tissue culture fluid		0	1	.973	.684	.399	
Modoc	tissue culture fluid		0	1	.153	.179	.185	

[a]Absorbance values for a given test sample were compared to the most similar control mosquito pool and the PBS-tween control. Those samples that were 3 standard deviations from their matched control are underscored "___" and 2 standard deviations are underscored "_ _ _".

upon thawing, were subjected to high speed centrifugation (Eppendorf, 15,600xg) for 1 min., sonification (Sonifer Cell Disruptor, 50 watts) in a -10°C ice bath for 30 sec., or both. Specificity was examined by testing pools of A. triseriatus infected with Jamestown Canyon virus, proven FA positive by the indirect method. Positive and negative controls were tissue culture fluids of LAC virus and Modoc virus, respectively.

LAC antigen was detected in as small a pool as 1 mosquito in 1.0 ml of diluent or 1 infected mosquito added to 50 non-infected mosquitoes in 1.0 ml of diluent (Table 48). The additional procedures of centrifugation and/or sonification did not appear to improve sensitivity (Table 49). Specificity of the LAC-antigen detection system appears reasonable, although this remains to be investigated further. ELISA seemed to be a sensitive and practical method for detection of arboviral antigen in mosquito preparations.

ELISA to detect Guaroa antigen in mosquito pools (K. Obom and R. Shope). Antigen detection studies using ELISA have continued on a bunyavirus (Guaroa) model system. The most sensitive procedure proved to be a four-layer sandwich method as opposed to a double-antibody method. Employing this procedure, as little as 6.6×10^4 plaque forming units (pfu) of virus were detected. The procedure detected Guaroa virus in a pool of mosquitoes containing 2 infected and 50 uninfected insects.

Monoclonal antibodies to Rift Valley fever viruses (J. Meegan). Monoclonal antibodies will be utilized not only for exploring the antigenic sites on proteins (or subunits) from Phlebotomus fever group viruses but also as a source of large quantities of purified antibodies for use with ELISA and other rapid diagnostic tests. Monoclonal antibodies are produced from hybrid cells formed by fusion of a myeloma cell line with spleen cells from an immunized mouse. Briefly, the method involves immunization of C57 BL/6 mice with 3-5 weekly doses of viral antigen mixed with equal volumes of adjuvant. Three days after the final booster spleens are removed from immunized mice and fused (using polyethylene glycol 1500) with BALB/C NSI/1 myeloma cells at a 4:1 ratio. In media containing hypoxanthine, aminopterin, and thymidine the parental myeloma cells are not viable. Parental non-fused spleen cells will not grow in cell culture. Thus, only hybridomas will grow and supernatants from these can be tested with IFA and ELISA tests for antibody production. Hybridomas producing specific antibodies can then be maintained in cell culture or inoculated i.p. in isogenic mice with the resulting ascitic fluids containing monoclonal antibody.

We have employed a number of different inactivated RVF virus antigens to immunize mice for hybridoma studies. Virus bands from ultracentrifuged (potassium tartrate-glycerol gradients) vaccine or mouse liver preparations as well as the unpurified starting materials were used in the ongoing experiments summarized in Table 50 . The ELISA test was employed for the initial screening of hybridomas and periodically during growth and passage. Table 50 includes results from our earliest trials which did not yield as many hybridomas as later fusions; and the percentage of positive wells is thus somewhat low. Further testing is ongoing to determine the

Table 49

The effect of high speed centrifugation and sonication on mosquito pools in the ELISA test[a]

Sample description				Percent change in absorbance values		
				dilution of sample		
Pool identification	No. infected	No. non-infected	Type of procedure	10^{o}	10^{-1}	10^{-2}
a	1	10	C	-6.36	-1.00	-9.54
a	1	10	C,S	-17.35	-8.02	-18.70
b	1	10	C	-39.10	-25.39	-1.34
c	1	50	C	-35.62	-22.91	-15.30
c	1	50	C,S	-30.88	-25.08	-22.76
d	3	50	C	-17.00	-14.31	-20.38
d	3	50	C,S	-17.61	-22.01	-35.50
e	3	50	C	-14.15	-25.72	-2.39
f	0	10	C	-7.41	-18.09	+8.57
f	0	10	C,S	+1.59	-6.91	-4.57
g	0	50	C	-24.56	-36.50	-1.08
g	0	50	C,S	-8.77	-34.60	-12.97

[a]pools subjected to sonification (S) followed by centrifugation (C) or just centrifugation.

specificity of the positive hybridomas and to clone all lines producing monoclonal antibodies.

Rhipicephalus appendiculatus tick cell line (LST-RA-243) for growth of Connecticut virus: need to passage infected mouse brain through cell culture to obtain susceptibility (B.Q.Chen and S.M.Buckley). Connecticut virus of the Sawgrass group was successfully grown in LST-RA-243 cells, but only from cell culture derived virus, not from infected mouse brain. Adaptation of LST-RA-243 cells to trypsinization and mass culture was described in the 1979 Annual Report (p.69). Infected mouse brain, passage 5, was used to inoculate BHK-21, Vero, and LST-RA-243 cells. CPE was noted in BHK-21 and Vero cells. In the LST-RA-243 cells there was neither CPE nor growth as determined on day 7 by subculture into BHK-21 cells. Three additional attempts to infect the tick cells with mouse brain were unsuccessful, however a single passage of Connecticut virus in BHK-21 or C6/36 Aedes albopictus cells resulted in virus stock capable of infecting the tick cells. The results are shown in Table 51.

On the possibility that either defective-interfering particles in mouse brain or simply a low-titered inoculum accounted for the inability to infect tick cells, a comparative titration of the mouse brain stock was done in C6/36 and LST-RA-243 cells. The results (Table 52) were identical whether a 1:10 or 1:100,000 dilution was used. Addition of 10% normal mouse brain to the BHK-21 inoculum did not inhibit infectivity for LST-RA-243 cells (Table 53). Growth of Connecticut virus from BHK-21 cells initially was slower in the tick cell line than in mosquito cells, however, by 72 hr the titer was 8.0 log $TCID_{50}$in both cells (Table 54). A persistent infection was established in the LST-RA-243 cells with titers ranging between 6.0 and 8.5 log $TCID_{50}$/ml for at least 21 days.

The explanation for the inability to infect tick cells with mouse brain-infected Connecticut virus is not clear. Perhaps the mouse selects against a virus population infective for tick cells. This phenomenon is important to remember when cell line susceptibility range is under consideration.

Replication of Tyuleniy virus in both tick and mosquito cell cultures (B.Q. Chen and S.M. Buckley). Tyuleniy virus has been isolated in nature from Ixodes putus ticks in the USSR and has also been shown by Soviet workers to be transmitted experimentally by mosquitoes. Four mosquito cell lines and the Rhipicephalus appendiculatus tick cell line were inoculated with 5.5 dex Tyuleniy virus of mouse brain origin. The supernatant fluids were assayed in BHK-21 cells on days 3, 7, 14, and 25 and cells were examined for immunofluorescence on day 25. Virus titered 2.0 and 3.5 $TCID_{50}$/ml on days 7 and 14 respectively in the tick cells. The virus also grew well in Aedes pseudoscutellaris cells, reaching a titer of 8.5 $TCID_{50}$/ml by day 25. Virus was not detected in Toxorhynchites amboinensis Ta 14a cells or Aedes aegypti C 17 cells. Virus was detected in undiluted fluid of T. amboinensis Ta 12A cells on day 14 only. Immunofluorescence was found in the tick cells and in A. pseudoscutellaris cells but not in the other mosquito cell lines. This is the first time in this laboratory that a tick-borne flavivirus has been demonstrated to replicate in a mosquito cell line.

Table 50. Development of hybridomas producing antibodies to RVF virus

RVF virus immunogen	# of wells seeded	# of wells yielding hybridomas	# of hybridomas positive in ELISA[1]	#of passaged hybridomas cell lines positive in ELISA[2]
Vaccine (Entebbe strain)	388	183	35	24
Vaccine-purified (Entebbe strain)	382	I.P.[3]	-	-
Mouse liver HA antigen purified (Entebbe strain)	302 288	182 179	72 I.P.	31 -
Mouse liver HA antigen (Zagazig 501 strain)	384	267	I.P.	-

[1]Tested twice during growth in 96 well plates.
[2]Tested three times during growth in 24 well plates and 6 well plates.
[3]I.P.= in progress.

Table 51

Susceptibility of BHK-21, Vero, LST-RA-243 and C6/36 cell lines to infection with Connecticut virus

Virus stock	Virus passage level	Number of experiment	Cell lines	Days after incubation	CPE	Dex TCD_{50}/ml
Mouse	5	1	BHK-21	7	+	8.5
Mouse	5	1	Vero	7	+	4.5
Mouse	5	1	LST-RA-243	7	0	<2.0
Mouse	5	2	BHK-21	7	+	9.5
Mouse	5	2	LST-RA-243	7	0	<2.0
Mouse	5	2	C6/36	7	0	6.5
Mouse	5	3	LST-RA-243	7	0	<2.0
Mouse	5	3	C6/36	7	0	6.7
Mouse	5	4	LST-RA-243	7	0	<2.0
BHK-21	1*	4	LST-RA-243	7	0	6.0
C6/36	1*	4	LST-RA-243	7	0	6.5

*One passage in BHK-21 or C6/36 cells following 5 mouse passages.

Table 52

Dose-response infection of LST-RA-243 and C6/36 cell lines with Connecticut virus

Virus stock	Virus passage level	Virus inoculum (dilution)*	Days after inoculation	Dex TCD_{50} per ml	
				C6/36	LST-RA-243
Mouse	5	1:10	7	8.4	<2.0
Mouse	5	1:100	7	8.7	<2.0
Mouse	5	1:1000	7	8.2	<2.0
Mouse	5	1:10,000	7	8.2	<2.0
Mouse	5	1:100,000	7	8.4	<2.0

*Elimination of possible truncated particles by serial dilution.

Table 53

Effect of normal mouse brain on infection of LST-RA-243 cells with Connecticut virus

Virus stock	Virus passage level	Treatment prior to virus adsorption	Days after inoculation	Dex TCD_{50}/ml
BHK-21	1	none	7	9.0
BHK-21	1	10% normal mouse brain	7	9.3

Table 54

Growth of Connecticut virus in C6/36 and LST-RA-243 cells

Source of virus	Cells	17	48	72
BHK-21	LST-RA-243	0*	7.5	8.0
BHK-21	C6/36	4.5	5.0	6.0
Mouse brain	LST-RA-243	0	0	0
Mouse brain	C6/36	6.0	6.0	8.0

*Log $TCID_{50}/ml$; $0 = < 2.0$

Application of the plaque reduction neutralization test (PRNT) to detect antibody induced by 17-D yellow fever vaccine during the 1978-79 Trinidad epidemic (M.J.Lewis, G.Roze, and W.G.Downs). During the yellow fever epidemic starting in November 1978, mass 17-D vaccination was carried out in Trinidad. Sera from vaccinees were sent to YARU for serological confirmation of efficacy of the program. PRNT with 17-D virus in Vero cells was done on 222 sera of vaccinees. One hundred nine were from residents of Port-of-Spain and 113 were from the rural area of Sangre Grande. Nine more were from persons vaccinated in 1976 and one in 1973. Several lots of vaccine from different countries-of-origin were represented in the sample.

Of 232 sera, all but 1 neutralized 17-D virus (Table 55) using a 50% reduction criterion. In test number 1 of Table 55, the criterion for neutralization was comparison with the positive control (Table 56) since the virus dose was apparently greater than planned.

The single non-reactor was from Sangre Grande and was said to have received vaccine manufactured in Brazil. Overall, 99.6% of vaccines had antibody, indicating a successful vaccination campaign from the sero conversion point-of-view.

Application of immunofluorescence and ELISA tests to detection of antibodies following immunization with 17D, yellow fever. (J.D. Converse, W.G. Downs, and J. Casals). The spot slides for IF tests were prepared with Asibi strain of yellow fever, in Vero cells. Antigen for ELISA tests was prepared from A. albopictus C6/36 cells infected with Asibi strain; the virus was precipitated with 10% polyethylene glycol 6000 in NaCl 2.3%. After overnight incubation at 4C, the suspension was centrifuged at 10,000 rpm for 1 hour. The pellet, resuspended in TRIS buffer at pH 7.3, was layered on a discontinuous sucrose gradient and centrifuged at 95000 g for 2 hours. The diffuse band was collected and titrated against a known system to determine the dilution at which it was to be used to cover the polystyrene plates. The ELISA test was carried out in the routine manner and the results read by means of an ELISA-READER (Titertek). End-points were calculated by interpolation, i.e., by comparison of the linear regression analysis slope of the test serum with the regression slope of the control serum, using dilutions 1:10 to 1:2560. The titer of a serum is expressed as the highest dilution which gave an O.D. (optical density) value of 0.1 or greater above the normal control.

Pre- and post-vaccination sera from 13 individuals were compared by plaque reduction test -- at 90% and 50% reduction --, hemagglutination inhibition, ELISA and IF; the results are given in Table 57. The conclusion from this study appears to be that following vaccination with 17D vaccine, neither ELISA nor IF tests detected antibodies, with few exceptions, in persons who were positive both by plaque reduction and HI tests. Furthermore, two of the 13 individuals who were strongly postive by ELISA following vaccination, were also positive with unchanged titers before vaccination.

Table 55

17-D yellow fever plaque counts in PRNT's of persons vaccinated in Trinidad, 1978-79

Plaque count	Test No. 1	2	3	4	5	6	7	8	9	10	11	12	13
0-10	0	8	8	15	17	11	16	15	8	13	9	4	16
11-20	1	3	4	2	5	3	1	4	5	5	6	2	5
21-30	1	0	2	0	0	2	1	2	2	5	2	0	1
31-40	2	1	2	2	1	1	1	2	1		0	0	0
41-50	0						1		1		2	1	1
51-60	3										1		
61-70	1												
71-80	0												
81-90	2												1[a]
91-100	0												
101-110	1												
Confluent				1[a]									

[a]Non-protective serum, test 4; repeated test 13.

Table 56

Plaque counts of negative and positive control antibody in PRNT's of Trinidad vaccinees

	Test No.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Negative control	conf[a]	conf	conf	conf	101	101	100	122	105	134	115	106	148
Positive control	81	27	11	14	15	17	9	20	8	20	8	13	23

[a]Number of plaques; conf=confluent destruction of cell sheet.

Crimean-Congo hemorrhagic fever in Iraq (J. Casals). Between September 16 and 25, 1980, J. Casals visited Baghad, Iraq as a WHO consultant on Crimean-Congo hemorrhagic fever (CCHF). He visited with Dr. Tantawi, University of Baghdad; Dr. Jurji, in charge of the Hemorrhagic Fevers Unit; Drs. Dabbagh, Muhedeen, Kirma, Al-Khoury, Al-Ani, and Hassan of Hummayad Fever Hospital. The gathered information is the subject of a report to WHO, the salient parts of which are given here.

The onset of the first recognized case of CCHF in Iraq was on September 3, 1979; the case occurred in Ramadi, about 100 Km west of Baghdad, where she died on September 9, 1979. Two hospital-acquired cases developed shortly after admission of the index case, a resident physician and an auxiliary nurse, both of whom died on September 19th. From that time until September 20, 1980, a total of 55 cases have been registered by the Office of the Director of Epidemiology and Quarantine (DEQ), Directorate General of Preventive Medicine, Ministry of Health.

Distribution of cases in time and space. Cases occurred sporadically throughout nearly a 12-month period, with increased accumulations in September-November, 1979, and April-June, 1980. Twenty-two cases occurred in the city of Baghdad and 33 in The Provinces. At least 2 of the Baghdad patients lived very near the hub of the city, Thahrir square; the others were located within a radius of from 7 to 10 Km of the city center.

The cases were singly and widely scattered in the capital and outside it, with no connection between any of them, with two exceptions, the index case and the two hospital-associated, secondary cases; and two sisters living in the same household who, judging from their admission dates were probably infected from the same source rather than one from the other. This distribution pattern bespeaks a widely distributed endemic situation in Iraq extending, at least, over the southern half of the area between the rivers.

Distribution of cases by sex and age. Twenty-three patients were males and 32 females; given the numbers involved the difference is not significant. The disease affected persons of all ages but was more frequently seen in youth and middle age: the number of cases in children (1-14 years) was 13; in adults, from 15 to 49 years, 35 cases; and in persons 50 years and older, 7 cases.

Clinical considerations. The clinical picture described to Dr. Casals by the clinicians at Fever Hospital was similar to that reported in other areas where the disease has occurred, USSR and Pakistan, but in its most severe form with prominent gastro-intestinal hemorrhages, extensive echymoses which in photographs seen during the consultation nearly covered the entire upper extremities and face and pronounced toxemia and dehydration; the severity of the disease is borne out by the fact that 35 of the 55 patients died and of these 35, 19 expired within 24 hours from hospital admission, probably between 6 and 8 days from onset. Death was generally attributed to shock and hepato-renal failure; complications such as pneumonia and septicemia were common.

Table 57

Antibody development following vaccination of man with 17-D yellow fever vaccine

Subject	Day bled after vaccination	Test, antigen, reciprocal of titer					
		PR		HI		ELISA	IF
		90%	50%	YF	JE		
CR	-1	0	0	0	0	0	0
	76	16-32	32+	20	0	0	4?
JG	-1	0	0	0	0	0	0
	43	16-32	32+	40	0	0	4?
GK	-7	0	0	0	0	0	0
	180	8-	32+	20	0	0	0
KO	-1	0	0	0	0	0	0
	270			40	0	0	0
SH	0			0	0	0	0
	15			40	0	80	0
BA	-6	0	0	0	0	320	0
	74	16	32+	40	0	320-640	0
	285			20	0	160-320	0
SB	0	0	0			0	
	75	8	32+	80+	10	0	4
MT	-2	0	0	0	0	0	0
	39	8-	16	40	0	0	0

Subject	Day bled after vaccination	Test, antigen, reciprocal of titer					
		PR		HI		ELISA	IF
		90%	50%	YF	JE		
JR	-1	0	0	0	0	320-640	0
	21	8-	32+	40	0	320	0
TT	0			0	0	0	0
	48			40	0	0	0
SH	-1	0	0	0	0	0	0
	790	8	32+	20	0	0	0
	870	8	32+	20	0	0	0
JK	?	0	8	20	0	0	4?
	?+28	16-32	32+	80+	0	0	4
MG	?	0	0	0	0	0	0
	?+197	32+	80+	0	0	0	0

PR=plaque reduction; HI=hemagglutination-inhibition; IF=immunofluorescence; YF=yellow fever; JE=Japanese encephalitis.
First dilution of serum: PR, 1:8; HI, 1:10; ELISA, 1:20; IF, 1:4.

Mode of infection. According to information from several sources--DEQ, Virology laboratory, physicians at Fever Hospital--few of the patients who lived long enough after admission to be questioned associated their disease with tick-bites or any bites: they did not observe ticks biting them shortly before falling ill. On the other hand, nearly all, or their families, mentioned close association with livestock in the household, mainly sheep and goats, fewer with cattle; no special mention was made of butchering livestock shortly before the onset of illness. Some of the field epidemiologists stated that it is conceivable that tick bites among people who keep livestock are so common that they are easily overlooked or, if mentioned in connection with the disease, they are unreliable.

A few domiciliary visits during the consultation clearly demonstrated not only the close association between patients and sheep or other livestock right in the city of Baghdad, but also the fact that tick bites had been noted and remembered by some of the patients.

Specific diagnosis, Virological. Attempts to isolate virus were made in 8 or 10 of the 55 patients; 4 patients yielded virus strains. Whether a fifth patient was positive could not be definitely established due to contradictory records. In all 4 instances, the strains were specifically identified as CCHF virus by means of CF and/or IF tests with a reference mouse hyperimmune reagent.

Serological diagnosis. Results of CF tests for serum antibodies were available for 17 of the 55 patients. Fifteen of the 20 survivors had antibodies with titers from 1:8 to 1:2048 and 5 were not tested; a fatal case tested on the day of admission was negative and a second one who died after 4 days in the hospital was positive with a titer of 1:32.

No results were made available to indicate whether the 15 positive survivors had actually showed serological conversion, i.e., a four-fold or greater increase in antibody titer between early and late sera. The results supplied were the titers observed between 3 and 4 weeks after hospital admission.

Accepting CF antibody titers of 1:128 and higher as indicative of recent infection one can accept as likely that 9 cases were serologically confirmed.

Summarizing the preceeding data it can be accepted that a specific virological and/or serological diagnosis was made in 11 instances.

Entomological aspects. Four small tick pools, species and stage undetermined, were tested at the virological laboratory by inoculation to cell cultures, in attempts to isolate virus. An additional pool was sent to Porton Down. No CCHF virus was isolated from these pools.

Serological surveys: Man. A survey carried out in 1979-1980 with sera from 1,580 persons from various parts of the country showed a high proportion with CF antibodies (Al-Tikriti et al., in preparation, 1981). Among patients' relatives and contacts, 29 percent of 257 were positive; 29

percent of 233 animal breeders in northern Iraq were positive; 7 percent of 773 abattoir workers and 10 percent of 166 hospital staffs were also positive. By contrast a random sample of 151 individuals not known to have had contact with cases were all negative.

Summary. Clinical and epidemiological observations from September 1979 to September 1980 lead to the conclusion that CCHF is endemic in Iraq from the valley of the Euphrates river eastward, particularly in the central and southern part of that area including the city of Baghdad. In addition, serological surveys with human sera show antibodies against the virus; in view of the known antigenic relationships of CCHF virus, these antibodies are most likely due to activity of the virus in the populations surveyed. The wide endemicity of the disease is borne out by the distribution of cases in time and space; there is no apparent connection between cases (excepting two nosocomial infections) and they are distributed over the entire year, with the exception of two winter months. These facts indicate multiple and unrelated foci of infection.

Major unanswered questions include: How is CCHF maintained in nature in Iraq? What tick(s) is involved in reservoir maintenance and transmission to man? Is virus transmitted from blood of livestock? Are the positive serological survey results specific?

YARU, through WHO, has supplied Iraq CCHF spot-slides for immunofluorescence tests and plans to continue to do so.

IX. COLLECTION OF LOW PASSAGE YELLOW FEVER REFERENCE STRAINS (R.B. Tesh).

One of the recommendations of the PAHO-sponsored Yellow Fever Symposium, held in Belem, Brazil in April 1980, was that an effort should be made to collect and preserve low-passage strains of yellow fever virus from various regions of Africa and the Americas for future reference. Accordingly, letters were sent to a number of virus laboratories located in areas where the virus is endemic. To date, 15 strains have been received. These are listed in Table 58. A stock of each virus strain has been prepared in cultures of Aedes albopictus cells (C6/36 clone) and has been lyophilized for storage. These specimens are available for distribution to interested investigators.

X. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (R.E. Shope, J. Casals, A. Main, S. Buckley, G.H. Tignor, J. Meegan).

The equivalent of 668 ampoules of arbovirus reagents were distributed from the WHO Centre to laboratories in 23 countries during 1980. This total consisted of 326 ampoules of virus stock, 136 ampoules of virus antigen, and 191 ampoules of mouse immune ascitic fluid or sera. Of the viruses and antibody distributed, there were represented 196 different arboviruses.

During 1980, the equivalent of 612 ampoules of arbovirus reagents was referred to this Centre from laboratories in 20 different countries. The

Table 58 - Low passage yellow fever virus strains

Strain no.	Passage history	Source	Locality	Date Collected	Contributors
Ar B 9005	SM_5, $MOSQ_1$	Aedes africanus	Bozo, Cent. Afr. Rep.	Nov. 3, 1977	J.J. Salaun, Institute Pasteur, Dakar
Ar B 8883	SM_5, $MOSQ_1$	Aedes africanus	Bozo, Cent. Afr. Rep.	Oct. 28, 1977	J.J. Salaun
Ar D 25865	SM_5, $MOSQ_1$	Aedes furcifer-taylori (males)	Kedougou, Senegal	Dec. 1977	J.J. Salaun
T 797984	$MOSQ._1$	human liver	Trinidad	1979	Barbara Hull, Caribbean Epidemiology Centre, Port of Spain, Trinidad
T 790882	$MOSQ_1$	Haemagogus janthinomys	Trinidad	1979	Barbara Hull
Ar 232869	SM_2, $MOSQ_1$	Haemagogus sp.	Brazil	March 3, 1973	Francisco Pinheiro, Instituto Evandro Chagas, Belem
Ar 350397	SM_2, $MOSQ_1$	Haemagogus sp.	Belterra, Para, Brazil	Aug. 11, 1978	Francisco Pinheiro
H 3509698	SM_2, $MOSQ_1$	human liver	Tome-Acu, Para, Brazil	Aug. 1978	Francisco Pinheiro
Be Ar 233164	$MOSQ_4$	Haemagogus sp.	Goias, Brazil	1973	Francisco Pinheiro
TRVL 4205	$Aotus_1$, $MOSQ_2$	liver of dead Alouatta seniculus	Trinidad	1954	Karl Johnson, Middle America Research Unit
79 H 327	$MOSQ_2$	human serum	Minteh Kunda, North Bank Div., Gambia	Jan. 12, 1979	Thomas Monath, Center for Disease Control, Ft. Collins, CO.

Strain no.	Passage history	Source	Locality	Date Collected	Contributors
P128M_c SMB/IVIC	SM_3, $MOSQ_1$	liver of Alouatta seniculus	Las Claveles, Cojedes, Venezuela	Oct. 1, 1959	Raul Walder, IVIC, Caracas, Venezuela
PHO-42H, SMB/IVIC-2	SM_2, $MOSQ_1$	human liver	San Rafael de el Pinal, Tachira, Venezuela	Nov 12, 1961	Raul Walder
Asibi	$Monkey_4$	human serum	Kpeve, Ghana	June 1927	R.E. Shope, YARU
R35740	SM_1, $MOSQ_1$	human liver	Ayacucho Dept.,Peru	Feb. 1979	C.H. Calisher, CDC Fort Collins, CO

referrals consisted of 278 viruses (Table 59), 276 ampoules of antigens, and 58 ampoules of immune reagents. In addition, 1,186 sera were received for arbovirus antibody survey testing.

Eight different cell lines were distributed in 1980. The lines and recipients are listed in Table 60.

Table 59

Virus referred to YARU for identification and study, 1980

Country of origin; strain	Source	Information from donor	YARU identification
Australia			
CSIRO 20		Eubenangee group	
23		Eubenangee group	
32		Eubenangee group	
33		Eubenangee group	
34		Eubenangee group	
36		Eubenangee group	
44		Wallal group	
12		Warrego group	
58		Palyam group	
82		Palyam group	
76		Corriparta group	
109		Corriparta group	
134		Corriparta group	
Brazil			
BeH-95781	Man	rabies	
Reg-20653		rabies	
Reg-20550		rabies	
Reg-20719		rabies	
Reg-20842		rabies	
Reg-20315		rabies	
Canada			
50-51 virus	Dermacentor variabilis		Ungrouped,probably new
Egypt			
EgAr 989	Culex pipiens		Sindbis
EgAr 996	Culex pipiens		Sindbis
EgAr 1003	Culex pipiens		Sindbis
EgAr 1019	Anopheles spp.		Sindbis

Table 59 (continued)

Virus referred to YARU for identification and study, 1980

Country of origin; strain	Source	Information from donor	YARU identification
EgAr 984	Culex pipiens		Not viable
EgAr 988	Culex pipiens		Not viable
EgAr 993	Culex pipiens		Not viable
EgAr 994	Culex pipiens		Not viable
France			
Brest Ar T222	Tick	Kemerovo group	Chenuda complex
Brest Ar T234	Tick	Hughes group	Subtype of Soldado
Gambia			
79 H 327		Yellow fever	
Netherlands			
Meuzelar	Man	Colorado Tick fever	Colorado tick fever

Table 59 (continued)

Virus referred to YARU for identification and study, 1980

Country of origin; strain	Source	Information from donor	YARU identification
New Caledonia			
NCAr 201	Aedes vigilax		Not viable
NCAr 204	Aedes vigilax		Not viable
NCAr 208	Aedes vigilax		Not viable
NCAr 238	Aedes vigilax		Not viable
NCAr 250	Coqui. xanthogaster	group B	dengue 4
NCAr 254	Aedes notoscriptus		group B
NCAr 259	Aedes vigilax		
NCAr 262	Aedes vigilax		group B
NCAr 264	Culex quinquefasciatus		group B
NCAr 265	Culex annulirostris		
NCAr 267	Aedes notoscriptus		
NCAr 280	Culex annulirostris		
NCAr 281	Culex annulirostris	group B	group B
NCAr 309	Aedes vigilax		
NCAr 310	Aedes vigilax		Not viable
NCAr 318	Aedes vigilax		
NCAr 323	Culex bitaeniorhynchus		group B
NCAr 329	Aedes aegypti		
NC AN 1	Corvus (bird)		
NC AN 28	Pterodroma leucoptera		Not viable

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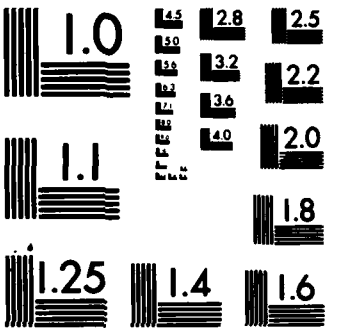


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

Table 59 (continued)

Virus referred to YARU for identification and study, 1980

Country of origin; strain	Source	Information from donor	YARU identification
Panama			
150 strains	Phlebotomines	Changuinola group	
	Aedes taeniorhynchus	California group,new	
	Aedes vittatus	California group,new	
Senegal			
Dak Ar Mg 802			
Dak Ar A 94			
Dak Ar D28542			
Dak Ar B2032			
Dak Ar B7343		group Bunyamwera,new	
Dak Ar B4653		group B	
Dak Ar B11266		group B	
South Africa			
Natal bat	bat	rabies	Lagos bat virus
SA An 24630	dog	West Nile	West Nile
SA Ar 19786		close to Uganda S	Uganda S
SA Ar 13089			
SA Ar 25140		close to Bagaza	
Trinidad			
33 yellow fever strains	man, mosquito, monkey	yellow fever	

Table 59 (continued)

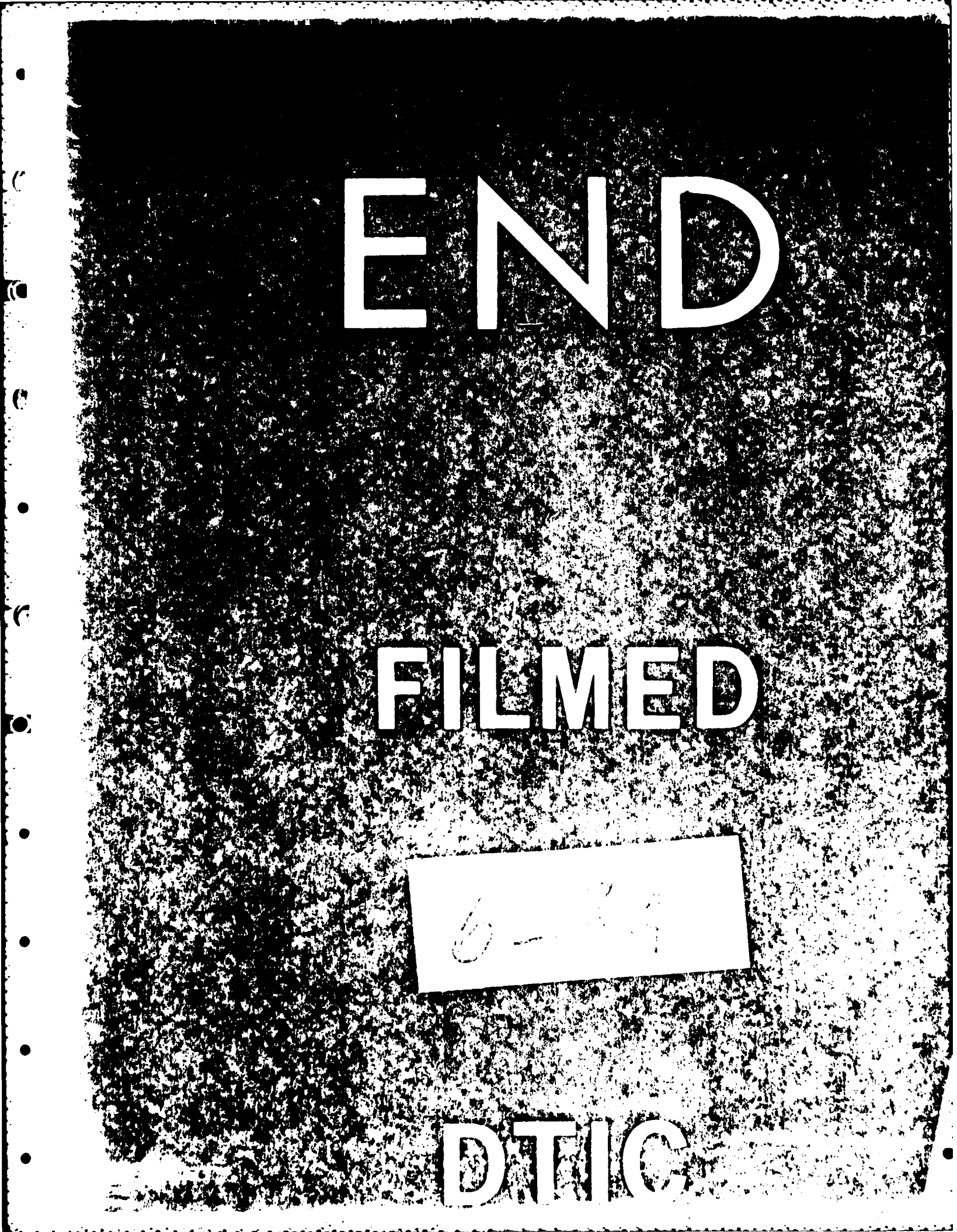
Virus referred to YARU for identification and study, 1980

Country of origin; strain	Source	Information from donor	YARU identification
USA			
CDC-1175	sentinel hamster		
1176	sentinel hamster		
10310	Cormorant		
10331	bird		
10333	bat		
10344	rodent		
16071	sentinel hamster		
16092	sentinel hamster		
16098	sentinel hamster		
16100	sentinel hamster		
16115	sentinel hamster		
16069	sentinel hamster		
16068	sentinel hamster		
10332	bird		
10312	man		
16111	sentinel hamster		
22 CTF strains	tick, man, rodent	CTF	
Yugoslavia			
E 544		rhabdovirus	Not viable
E 545		rhabdovirus	Not viable
E 546		rhabdovirus	Not viable

Table 60

Distribution of cell cultures during 1980

Investigator	Institution	BHK-21	XTC-2	C6/36	LST-MOS 61	LST-RA-243	RML-15
B.A.Baxton	Auburn University,Auburn,Alabama			X	X		
Dr.J.D.Converse	U.S.Naval Med.Res. Unit, Jakarta		X	X	X		X
C.Davidson	Universite de Montreal	X					
Dr.M.Jozan-Work	University of California,Los Angeles	X		X	X		
D. Lundino	W.Alton Jones Cell Science Center, Lake Placid			X	X		X
Dr.Li Ho-min	Ministry of Health,Beijing,China				X		
Dr.G.Oro	Gorgas Mem.Lab.,APO Miami,Florida			X			
Dr.G.Panon	Institut Pasteur,De Noumea			X	X		
Dr.W.A.Rowley	Iowa State University of Science and Techn., Ames, Iowa		X				
Dr.R.B.Tesh	Pacific Research Unit,Honolulu,Hawaii				X		
Dr. J. Thorsen	Vet.Microbiology and Immunology, Guelph, Ontario			X	X	X	X
Dr. R. Walder	Caracas			X			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